UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
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Ref: 8EPR-SR

February 1, 2010

MEMORADUM

SUBJECT: Modifications to the "Final Remedial Investigation for Operable Unit 3, Libby Asbestos Superfund Site, Phase III Sampling and Analysis Plan" dated May 26, 2009

FROM: Bonnie Lavelle, Remedial Project Manager
Libby Asbestos Sit, Operable Unit 3

TO: Libby Asbestos Site Operable Unit 3
Site File

The attached EPA-approved documents which include standard operating procedures, modifications to the text, field modifications and field reconnaissance information, constitute all modifications to the subject document.

FIELD MODIFICATION APPROVAL FORM
LFM-OU3-1
Libby OU3 Phase III Sampling & Analysis Plan

Requested by: Bonnie Lavelle Date: July 1, 2009

Description of Deviation:

Based on a field reconnaissance trip to OU3 on June 16-17, 2009, the script for the activity based sampling is modified to better represent likely activities under a current and future recreational use scenario. The revised script, Attachment A to the Phase III SAP, is attached to this field modification form.

☑ EPA Region 8 has reviewed this field modification and approves as proposed.

☐ EPA Region 8 has reviewed this field modification and approves with the following exceptions:

☐ EPA Region 8 has reviewed this field modification and does not agree with the proposed approach for the following reasons:

Bonnie Lavelle, EPA RPM Date 7/1/09

REVISION 1

LIBBY SUPERFUND SITE
OPERABLE UNIT 3
PHASE III SAP ATTACHMENT A

SCRIPT FOR COMPOSITE ABS FOR RECREATIONAL VISITOR SCENARIO

Each ABS sample will span a time period of 140 minutes. Each ABS sample will be a composite of activities that are considered to be representative of activities that are likely to be performed by recreational visitors to OU3 currently and in the future. This includes individuals who visit the area to hike, hunt, or camp. Two participants will participate in each ABS event. Table A-1 summarizes the activities and the timing. A more detailed description is provided below.

Activities

1. ATV Riding
The first activity to be performed is ATV riding. The vehicles will be Polaris Ranger 500® 4WD "side by side" or alternate vehicles of similar size and design. The total duration is 40 minutes. Riding should occur primarily on USFS roads or trails in the designated area, but should include off-trail riding if the terrain and forest density is suitable. Speed should be about 3-10 mph, depending on terrain and safety considerations. For the first 20 minutes, Person 1 should be in the lead position, with Person 2 following at a safe distance (about 5-10 meters, depending on terrain). After 20 minutes, Person 2 should take the lead, and Person 1 should follow at a safe distance.

2. Hiking
The second activity to be performed is hiking. The total duration is 40 minutes. Hiking should occur on trails (if present), and should also occur off-trail if the terrain is suitable. For the first 20 minutes, Person 1 should be in the lead position, with Person 2 following at a distance of about 3-5 meters, depending on terrain. After 20 minutes, Person 2 should take the lead, and Person 1 should follow.

3. Collecting Firewood
The third activity is collecting firewood for a campfire. This includes picking up dead wood small enough to start a campfire and also wood in the 2-8 inch size range that is lying on the forest floor, breaking the wood into pieces suitable for a campfire, and stacking the wood in a pile. Each participant will harvest firewood 20 minutes total.

4. Digging to prepare a campfire site
The fourth activity is digging. This is intended to simulate activities that bring a person into direct contact with soil and duff such as when preparing a campfire area. This activity may be

performed in a clearing in the forest or in a meadow area, depending on the local conditions and terrain.

For digging, each participant shall use a small camp shovel to dig a fire pit in the center of a suitable area. Both individuals shall be digging at the same time. If the area is mainly rock or meadow rather than forest, each individual shall collect rocks and use these to build a rock fire circle in the center of the area. Total time spent in this activity shall be 10 minutes.

5. Building and Sitting Near a Campfire

The final activity in the ABS script is building and sitting near a campfire. Fore safety reasons, this component of the script will not be performed in the ABS study area in the forest, but rather will be performed on W.R. Grace-owned property near Rainy Creek Road and Highway 37 (the area formerly known as the Flyway) in an area that has been specifically prepared to accommodate safe fire burning. In order to achieve this final element of the ABS script, the following steps are needed:

1. A representative set of firewood, collected in Step 3 (above), will be placed into a bag.
2. Each person shall turn off their air monitoring pump.
3. The collected firewood shall be transported by truck to the specified burning area.
4. Both individuals shall turn their air monitoring pumps back on. Then, both individuals should participate in building and lighting the fire. Once the fire is lit, both individuals should stand near the fire, simulating the activities of recreational campers. Each individual should move about the fire, including brief intervals of passing through the downwind direction, so that exposures from all wind angles are included in the composite sample.

After a total of 30 minutes, the fire will be thoroughly extinguished with water. This will end the ABS script and both air monitoring pumps shall be turned off.

Equipment decontamination. All non-disposable equipment, including ATVs, saws, rakes and shovels used during the investigation will be decontaminated between each ABS event using a pressurized water to remove accumulated material.

Each fire will be built in a steel pan that cam be decontaminated between fires by thorough rinsing with water.

Health and Safety. Each person who participates in ABS sampling shall wear sufficient personal protective equipment to ensure that unacceptable exposure to asbestos does not occur. In addition, all ABS related activities must be performed in fashion that ensures the safety of both individuals in the ABS team.

TABLE A-1
SUMMARY OF ABS AT OU3

Time (min)		Person	
Start	Stop	No. 1	No. 2
0	20	ATV (lead)	ATV (follow)
20	40	ATV (follow)	ATV (lead)
40	60	Hike (lead)	Hike (follow)
60	80	Hike (follow)	Hike (lead)
80	100	Collect wood for campfire	
100	110	Dig	Dig
110	140	Build and stand near campfire (a)	

(a) This activity is not performed in the ABS study area in the forest, but in a special area located in the Flyway.

FIELD MODIFICATION APPROVAL FORM
LFM-OU3-__2_
Libby OU3 Phase III Sampling & Analysis Plan

Requested by:___John Garr, MWH_____ Date:___7-13-09_____

Description of Deviation: As learned during the first round of ABS at OU3 during the week of July 6 through 10, 2009, the SKC QT-30 air sampling pumps maintain the set flow rate to within 2.5 % of the initial calibrated rate. In most instances, the flow rate at the beginning of the 110-minute forest script was identical to that at the end of the script. The greatest deviation observed was 0.2 LPM (2.5%), well within the +/- 5% specified in the SAP. To reduce interruptions to measure flow rate after each component activity of the script and to reduce the burden on those performing the script in Level C Modified, MWH suggests the flow rates be measured only at the beginning and end of the forest script and at the beginning and end of the wood-burning activity.

☑ EPA Region 8 has reviewed this field modification approves as proposed.

☐ EPA Region 8 has reviewed this field modification and approves with the following exceptions:

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☐ EPA Region 8 has reviewed this field modification and does not agree with the proposed approach for the following reasons:

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Bonita Lavelle, EPA RPM

7-13-09
Date

FIELD MODIFICATION APPROVAL FORM
LFM-OU3-3
Libby OU3 Phase III Sampling & Analysis Plan

Requested by: John Garr, MWH Date: July 15, 2009

Description of Deviation:

Attached is a revised FSDS for activity based sampling personal air monitoring. It has been simplified to match the revised flow rate measurements and a space has been added for calculating volumes. The form indicates the sample time for the forest script and the fire script. If actual times deviate from these it will be noted in the volume calculation on the form.

☑ EPA Region 8 has reviewed this field modification and approves as proposed.

☐ EPA Region 8 has reviewed this field modification and approves with the following exceptions:

☐ EPA Region 8 has reviewed this field modification and does not agree with the proposed approach for the following reasons:

Bonita Lavelle, EPA RPM

7-15-09
Date

LIBBY OU3 FIELD SAMPLE DATA SHEET
ACTIVITY-BASED SAMPLING (ABS) PERSONAL AIR MONITORING

ABS Area: ABS-_____ **Sampling Date: _____** **Sampling Team:** MWH

Person #1 Name: _____ Index ID: *AFFIX LABEL HERE*

Person #2 Name: _____ Index ID: *AFFIX LABEL HERE*

Field Blank Index ID: *AFFIX LABEL HERE* **Cassette Lot Number: 17826**

Field Logbook Number: _____ Field Logbook Pages: _____

Activity		Activity Sample Time (hh:mm)		Rotameter Flow Rate			
				Person #1		Person #2	
		Start	Stop	Start	Stop	Start	Stop
ATV	#1 Lead						
	#2 Lead						
Hiking	#1 Lead						
	#2 Lead						
Wood Gathering							
Digging							
Fire				_____LPM	_____LPM	_____LPM	_____LPM

Person #1 Pump ID No.: _____ Rotameter ID: _____ GPS ID: _____

Person #2 Pump ID No.: _____ Rotameter ID: _____ GPS ID: _____

Field Comments:

Weather Description--

Other--

VOLUME CALCULATION

#1 Ave. Flow Rate (Forest Script) =_____LPM x 110 Min. = _____ L #1 Ave. Flow Rate (Fire) = _____ LPM x 30 Min. = _____ L **SUM=**_____ L

#2 Ave. Flow Rate (Forest Script) =_____LPM x 110 Min. = _____ L #2 Ave. Flow Rate (Fire) = _____ LPM x 30 Min. = _____ L **SUM=**_____ L

NOTE: *USE ACTUAL TIMES FOR VOLUME CALCULATIONS*

Field Data Entered by:		**Field Entries Checked by:**	

Database Entry:		*Database QC:*

FIELD MODIFICATION APPROVAL FORM
LFM-OU3-4
Libby OU3 Phase III Sampling & Analysis Plan

Requested by: Bonita Lavelle, EPA Remedial Project Date: July 24, 2009

Description of Modification:

To minimize the possibility of overloading the filters during ABS, the ABS script has been modified to require collecting separate samples during the ATV riding activity and the remaining activities. The revised script is attached to this modification form.

In addition:

- The **target sensitivity** described in Section 3.1.4, page 20 of the final Phase III Sampling and Analysis Plan (Phase III SAP) is modified to **0.006 cc^{-1}** ; and
- The **target pump flow rate** described in Section 3.1.5, page 23 of the Phase III SAP is modified to **6.5 liters per minute for the ATV riding activity** and the target pump flow rate is maintained at **8 liters per minute for the remaining ABS activities**; and
- The stopping rules for asbestos analysis described in Section 3.1.6, page 25 of the Phase III SAP are modified as follows:
  For field samples, evaluate each sample until one of the following is achieved:
  - A minimum of 2 grid openings (GOs) in each of 2 grids has been examined.
  - The target sensitivity (0.006 cc^{-1}) is achieved. Assuming that the sample volumes will be between 260 liters (sample collected during ATV riding) and 800 liters (sample collected during remaining activities), that the sample may be analyzed using direct preparation, and that the area of a GO is 0.01 mm^2, it is expected that the target analytical sensitivity can be achieved by counting 25 GOs (sample collected during ATV riding) and 9 GOs (sample collected during remaining activities).
  - 50 LA structures are observed.
  - An area of 0.5 mm^2 has been examined (approximately 50 GOs).

☒ EPA Region 8 has reviewed this field modification and approves as proposed.

☐ EPA Region 8 has reviewed this field modification and approves with the following exceptions:

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☐ EPA Region 8 has reviewed this field modification and does not agree with the proposed approach for the following reasons:

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Bonita Lavelle, EPA RPM

7/24/09
Date

REVISION 2
July 24, 2009

LIBBY SUPERFUND SITE
OPERABLE UNIT 3
PHASE III SAP ATTACHMENT A

SCRIPT FOR COMPOSITE ABS FOR RECREATIONAL VISITOR SCENARIO

Each ABS event will span a time period of 140 minutes. Each ABS scenario consists of several activities that are considered to be representative of activities that are likely to be performed by recreational visitors to OU3 currently and in the future. This includes individuals who visit the area to hike, hunt, or camp. Two participants will participate in each ABS event. Tables A-1 and A-2 summarize the activities and the timing. For each ABS event at each location, two samples will be collected during the ATV riding activity and a separate set of two samples will be collected during the remaining activities. A more detailed description is provided below.

Activities

1. ATV Riding

The first activity to be performed is ATV riding. The vehicles will be Polaris Ranger 500® 4WD "side by side" or alternate vehicles of similar size and design. The total duration is 40 minutes. Riding should occur primarily on USFS roads or trails in the designated area, but should include off-trail riding if the terrain and forest density is suitable. Speed should be about 3-10 mph, depending on terrain and safety considerations. The ATVs should be ridden on the longest stretch of road possible within the designated ABS locations. The stretch of road may include up to ½ mile outside the designated ABS locations in either direction. For the first 20 minutes, Person 1 should be in the lead position, with Person 2 following. Distance between riders will be maintained based on terrain, visibility, and safety considerations. The trailing rider should be at a safe distance from the lead rider, close enough to be in the dust cloud of the lead rider, but not so close as to experience unreasonably high dust exposures. The objective is to simulate reasonable maximum exposure to ATV riders. After 20 minutes, Person 2 should take the lead, and Person 1 should follow at a safe distance. After 40 minutes of ATV riding, the air sampling pumps should be turned off and the samples submitted for analysis in accordance with the Phase III Sampling and Analysis Plan (SAP).

2. Hiking

The second activity to be performed is hiking. Collection of new air samples will begin at the start of the hiking activity. Sampling pumps should be fitted with new sample cassettes and turned on at the start of hiking. The total duration of hiking is 40 minutes. Hiking should occur on trails (if present), and should also occur off-trail if the terrain is suitable. For the first 20 minutes, Person 1 should be in the lead position, with Person 2 following at a distance of about 3-5 meters, depending on terrain. After 20 minutes, Person 2 should take the lead, and Person 1 should follow.

3. Collecting Firewood

The third activity is collecting firewood for a campfire. This includes picking up dead wood small enough to start a campfire and also wood in the 2-8 inch size range that is lying on the forest floor, breaking the wood into pieces suitable for a campfire, and stacking the wood in a pile. Each participant will harvest firewood 20 minutes total.

4. Digging to prepare a campfire site

The fourth activity is digging. This is intended to simulate activities that bring a person into direct contact with soil and duff such as when preparing a campfire area. This activity may be performed in a clearing in the forest or in a meadow area, depending on the local conditions and terrain.

For digging, each participant shall use a small camp shovel to dig a fire pit in the center of a suitable area. Both individuals shall be digging at the same time. If the area is mainly rock or meadow rather than forest, each individual shall collect rocks and use these to build a rock fire circle in the center of the area. Total time spent in this activity shall be 10 minutes.

5. Building and Sitting Near a Campfire

The final activity in the ABS script is building and sitting near a campfire. Fore safety reasons, this component of the script will not be performed in the ABS study area in the forest, but rather will be performed on W.R. Grace-owned property near Rainy Creek Road and Highway 37 (the area formerly known as the Flyway) in an area that has been specifically prepared to accommodate safe fire burning. In order to achieve this final element of the ABS script, the following steps are needed:

1. A representative set of firewood, collected in Step 3 (above), will be placed into a bag.
2. Each person shall turn off their air monitoring pump.
3. The collected firewood shall be transported by truck to the specified burning area.
4. Both individuals shall turn their air monitoring pumps back on. Then, both individuals should participate in building and lighting the fire. Once the fire is lit, both individuals should stand near the fire, simulating the activities of recreational campers. Each individual should move about the fire, including brief intervals of passing through the downwind direction, so that exposures from all wind angles are included in the composite sample.

After a total of 30 minutes, the fire will be thoroughly extinguished with water. This will end the ABS script and both air monitoring pumps shall be turned off and the air samples submitted for analysis in accordance with the Phase III SAP.

Equipment decontamination. All non-disposable equipment, including ATVs, saws, rakes and shovels used during the investigation will be decontaminated between each ABS event using a pressurized water to remove accumulated material.

Each fire will be built in a steel pan that cam be decontaminated between fires by thorough rinsing with water.

Health and Safety. Each person who participates in ABS sampling shall wear sufficient personal protective equipment to ensure that unacceptable exposure to asbestos does not occur. In addition, all ABS related activities must be performed in fashion that ensures the safety of both individuals in the ABS team.

TABLE A-1
SUMMARY OF ABS AT OU3
FIRST TWO SAMPLES FOR EACH EVENT

Time (min)		Person	
Start	Stop	No. 1	No. 2
0	20	ATV (lead)	ATV (follow)
20	40	ATV (follow)	ATV (lead)

TABLE A-2
SUMMARY OF ABS AT OU3
SECOND TWO SAMPLES FOR EACH EVENT

Time (min)		Person	
Start	Stop	No. 1	No. 2
0	20	Hike (lead)	Hike (follow)
20	40	Hike (follow)	Hike (lead)
40	60	Collect wood for campfire	
60	70	Dig	Dig
70	100	Build and stand near campfire (a)	

(a) This activity is not performed in the ABS study area in the forest, but in a special area located in the Flyway.

FIELD MODIFICATION APPROVAL FORM
LFM-OU3-5
Libby OU3 Phase III Sampling & Analysis Plan

Requested by: Bonita Lavelle, EPA Remedial Project Date: August 31, 2009

Description of Modification:

A. The script for the activity based sampling has been modified to better represent activities under a current and future recreational user scenario and to minimize the potential for overloading sample filters. The revised script, Revision 3 of Attachment A to the Phase III SAP, is attached to this field modification form.

B. In addition, the stopping rules for asbestos analysis of filters collected under the activity based sampling program portion of the Phase III Sampling and Analysis Plan are modified as follows:

- The **target sensitivity** is **0.006 cc^{-1}**;
- For field samples, evaluate each sample until one of the following is achieved:
  - A minimum of 2 grid openings (GOs) in each of 2 grids has been examined.
  - The target sensitivity (0.006 cc^{-1}) is achieved. (Assuming that the sample volumes will be between 40 liters and 80 liters for samples collected during ATV riding, 160 liters and 320 liters for samples collected during hiking, and 70 liters and 140 liters for samples collected during fire building and burning, that the samples may be analyzed using direct preparation, and that the area of a GO is 0.01 mm^2, it is expected that the target analytical sensitivity can be achieved by counting between 20 GOs and 160 GOs.)
  - 50 LA structures are observed.

C. This modification also institutes the following sample handling procedures:

- Sample cassettes will be stored in the boxes they were shipped in to maintain them in an upright position and will not be stored in plastic bags (which may be electrostatically charged).
- Exposed sample cassettes will be stored and transported with the open end of the cowl facing up and will be cushioned against vibration during transport. The cassettes will be shipped to the analytical laboratory in a cooler with a handle on top. The handle will prevent the cooler from being shipped upside down and will help ensure the cassettes remain vertical with the open end of the cowl facing up.

☒ EPA Region 8 has reviewed this field modification and approves as proposed.

☐ EPA Region 8 has reviewed this field modification and approves with the following exceptions:

☐ EPA Region 8 has reviewed this field modification and does not agree with the proposed approach for the following reasons:

Bonita Lavelle, EPA RPM Date 9/1/09

REVISION 3
August 31, 2009

LIBBY SUPERFUND SITE
OPERABLE UNIT 3
PHASE III SAP ATTACHMENT A

REVISED SCRIPT FOR COMPOSITE ABS SCENARIO

Each ABS event will span a sampling time period of 135 minutes. Each ABS scenario consists of several activities that are considered to be representative of activities that are likely to be performed by recreational visitors to OU3 currently and in the future. This includes individuals who visit the area to hike, hunt, or camp. Two participants will participate in each ABS event. Two pumps will be worn by each participant. Target flow rate for the first pump is 2 liters per minute (LPM) and the target flow rate for the second pump is 4 LPM. For each ABS event at each location, samples will be collected separately during the ATV riding activity, the hiking activity, and the combination of collecting firewood, digging to prepare a campfire site, and building and sitting near a fire. Table A-1 summarizes the activities and the timing. A more detailed description is provided below.

Activities

1. ATV Riding
The first activity to be performed is ATV riding. The vehicles will be Polaris Ranger 500® 4WD "side by side" or alternate vehicles of similar size and design. The total duration is 20 minutes. Riding should occur on USFS roads or trails in the designated area. Speed should be about 3-10 mph, depending on terrain and safety considerations. For the first 10 minutes, Person 1 should be in the lead position, with Person 2 following at a safe distance. The following distance should be far enough to be safe and should be approximately that which an ATV rider *without* PPE would follow (i.e., far enough behind the lead ATV that dust generated by the lead ATV has cleared sufficiently that the following rider is comfortable without respiratory protection). After 10 minutes, Person 2 should take the lead, and Person 1 should follow at a safe and appropriate distance.

2. Hiking
The second activity to be performed is hiking. The total duration is 80 minutes. Hiking should occur on trails (if present), and should also occur off-trail if the terrain is suitable. For the first 40 minutes, Person 1 should be in the lead position, with Person 2 following at a distance of about 3-5 meters, depending on terrain. After 40 minutes, Person 2 should take the lead, and Person 1 should follow.

3. Collecting Firewood
The third activity is collecting firewood for a campfire. This includes picking up dead wood small enough to start a campfire and also wood in the 2-8 inch size range that is lying on the

forest floor, breaking the wood into pieces suitable for a campfire, and stacking the wood in a pile. Each participant will harvest firewood 10 minutes.

4. Digging to prepare a campfire site

The fourth activity is digging. This is intended to simulate activities that bring a person into direct contact with soil and duff such as when preparing a campfire area. This activity may be performed in a clearing in the forest or in a meadow area, depending on the local conditions and terrain.

Each individual shall dig a separate fire pit (i.e., 2 pits will be dug, one by person 1 and one by person 2). Each participant shall use a small camp shovel to dig their fire pit in the center of a suitable area. The digging rate shall be such a realistic amount of dust generated will be generated (i.e., participants would feel comfortable digging without respiratory protection). If the area is mainly rock or meadow rather than forest, each individual shall collect rocks and use these to build a rock fire circle in the center of the area. Total time spent in this activity shall be 5 minutes.

5. Building and Sitting Near a Campfire

The final activity in the ABS script is building and sitting near a campfire. Fore safety reasons, this component of the script will not be performed in the ABS study area in the forest, but rather will be performed on W.R. Grace-owned property near Rainy Creek Road and Highway 37 (the area formerly known as the Flyway) in an area that has been specifically prepared to accommodate safe fire burning. In order to achieve this final element of the ABS script, the following steps are needed:

1. A representative set of firewood, collected in Step 3 (above), will be placed in a bag and placed onto the ATV.
2. Each person shall turn off their air monitoring pump.
3. The collected firewood shall be transported by ATV to the specified burning area.
4. Both individuals shall turn their air monitoring pumps back on. Then, both individuals should participate in building and lighting the fire. This is expected to take about 5 minutes. Once the fire is lit, both individuals should sit a safe distance from the fire, simulating the activities of recreational campers. Each individual should move about the fire periodically so as to simulate activities of recreational campers.

After a total of 20 minutes, the fire will be thoroughly extinguished with water. This will end the ABS script and both air monitoring pumps shall be turned off.

Equipment decontamination. All non-disposable equipment, including ATVs, saws, rakes and shovels used during the investigation will be decontaminated between each ABS event using a pressurized water to remove accumulated material.

Each fire will be built in a steel pan that cam be decontaminated between fires by thorough rinsing with water.

Health and Safety. Each person who participates in ABS sampling shall wear sufficient personal protective equipment to ensure that unacceptable exposure to asbestos does not occur. In addition, all ABS related activities must be performed in fashion that ensures the safety of both individuals in the ABS team.

TABLE A-1
SUMMARY OF ABS AT OU3

Scenario 1: ATV Riding

Time (min)		Person	
Start	Stop	No. 1	No. 2
0	10	ATV (lead)	ATV (follow)
10	20	ATV (follow)	ATV (lead)

Scenario 2: Hiking

Time (min)		Person	
Start	Stop	No. 1	No. 2
0	40	Hike (lead)	Hike (follow)
40	80	Hike (follow)	Hike (lead)

Scenario 3: Fire Building/Burning

Time (min)		Person	
Start	Stop	No. 1	No. 2
0	10	Collect wood for campfire	
10	15	Dig	Dig
15	20	Build and light fire	
20	35	Build and stand/sit near campfire	

FIELD MODIFICATION APPROVAL FORM
LFM-OU3-6
Libby OU3 Phase III Sampling & Analysis Plan

Requested by: Lynn Woodbury, SRC Date: 8/28/09

Description of Deviation:
A procedure for disinfecting vials containing small mammal tissues has been added.

In SOP MAMMAL-LIBBY-OU3 (Rev. 1), the first paragraph of page 11 of 17, Section 4.10 *Collection and Preparation of Tissue Samples* is replaced with the following:

> For each sample collected for potential future asbestos tissue burden analysis, the sample will be placed in a pre-numbered and pre-weighed (by EMSL) glass scintillation vial. The vial will contain no fluid. The vial number, the field ID number for the source animal, and the tissue type placed into each numbered vial will all be recorded on the FSDS. **After the tissue sample has been placed into the vial, tighten the cap, and immerse the vial in a 10% solution of bleach. Rinse the vial in clean water.** All vials with tissue samples will be maintained on wet ice until delivered to EMSL in Libby, MT for final weighing and storage for potential future tissue analysis.

☒ EPA Region 8 has reviewed this field modification approves as proposed.

☐ EPA Region 8 has reviewed this field modification and approves with the following exceptions:

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☐ EPA Region 8 has reviewed this field modification and does not agree with the proposed approach for the following reasons:

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Bonita Lavelle, EPA RPM

9/1/09
Date

FIELD MODIFICATION APPROVAL FORM
LFM-OU3-7
Libby OU3 Phase II Sampling & Analysis Plan

Requested by: John Garr, MWH Date: August 26, 2009

Description of Deviation:

The field sample data sheet (FSDS) for activity based sampling (ABS) personal air monitoring samples has been revised to be consistent with the latest revision of the ABS script (see field modification LFM-OU3-5 for the ABS script).

The revised FSDS is attached to this field modification form.

☒ EPA Region 8 has reviewed this field modification and approves as proposed.

☐ EPA Region 8 has reviewed this field modification and approves with the following exceptions:

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☐ EPA Region 8 has reviewed this field modification and does not agree with the proposed approach for the following reasons:

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Bonita Lavelle, EPA RPM Date 9/4/09

Sheet No.: P3-_____

LIBBY OU3 FIELD SAMPLE DATA SHEET

ACTIVITY-BASED SAMPLING (ABS) PERSONAL AIR MONITORING

ABS Area: ABS-_____ Sampling Date: _____ Sampling Team: MWH Field Logbook: _____ Pgs: _____

		ATV	HIKING	WG/FP/BU
Person #1: _____	(4.0 Lpm)	*AFFIX ATV LABEL HERE*	*AFFIX HIKING LABEL HERE*	*AFFIX WG/FP/BU LABEL HERE*
	(2.0 Lpm)	*AFFIX ATV LABEL HERE*	*AFFIX HIKING LABEL HERE*	*AFFIX WG/FP/BU LABEL HERE*
Person #2: _____	(4.0 Lpm)	*AFFIX ATV LABEL HERE*	*AFFIX HIKING LABEL HERE*	*AFFIX WG/FP/BU LABEL HERE*
	(2.0 Lpm)	*AFFIX ATV LABEL HERE*	*AFFIX HIKING LABEL HERE*	*AFFIX WG/FP/BU LABEL HERE*

Field Blank ID: *AFFIX LABEL HERE*

Cassette Lot Number: _____

Activity		Activity Sample Time (hh:mm)		Rotameter Flow Rate (Lpm)							
				Pump____		Pump____		Pump____		Pump____	
		Start	Stop	Start	Stop	Start	Stop	Start	Stop	Start	Stop
ATV	#1 Lead										
	#2 Lead										
Hiking	#1 Lead										
	#2 Lead										
Wood Gathering											
Digging											
Fire											

Person #1: 4.0 Lpm Pump ID: _____ 2.0 Lpm Pump ID: _____ Rotameter ID: _____ GPS ID: _____

Person #2: 4.0 Lpm Pump ID: _____ 2.0 Lpm Pump ID: _____ Rotameter ID: _____ GPS ID: _____

Field Comments:
Weather Description--

Other--

VOLUME CALCULATION Person #1

4L Ave. Flow Rate (ATV) =_____LPM x ____ Min. = _____ L

2L Ave. Flow Rate (ATV)= _____ LPM x ____ Min. = _____ L

4L Ave. Flow Rate (Hiking) =_____ LPM x ___ Min. = _____ L + 4L Ave. Flow Rate (WG/FP/BU) = _____ LPM x ___ Min. = _____ L

2L Ave. Flow Rate (Hiking) =_____ LPM x ___ Min. = _____ L + 2L Ave. Flow Rate (WG/FP/BU) = _____ LPM x ___ Min. = _____ L

VOLUME CALCULATION Person #2

4L Ave. Flow Rate (ATV) =_____LPM x ____ Min. = _____ L

2L Ave. Flow Rate (ATV)= _____ LPM x ____ Min. = _____ L

4L Ave. Flow Rate (Hiking) =_____ LPM x ___ Min. = _____ L + 4L Ave. Flow Rate (WG/FP/BU) = _____ LPM x ___ Min. = _____ L

2L Ave. Flow Rate (Hiking) =_____ LPM x ___ Min. = _____ L + 2L Ave. Flow Rate (WG/FP/BU) = _____ LPM x ___ Min. = _____ L

Field Data Entered by:		Field Entries Checked by:	

Database Entry:		*Database QC:*

Date: June 3, 2009 SOP# HISTO-LIBBY-OU3 (Rev. 1)

Title: SMALL MAMMAL TISSUE HISTOLOGY

APPROVALS

TEAM MEMBER	SIGNATURE/TITLE	DATE
EPA Remedial Project Manager		8/17/09
SOP Author (Parametrix)		8/10/09
SOP Author (Northwest ZooPath)		8/10/09

Revision Number	Date	Reason for Revision
0	06/03/2009	--
1	8/10/09	Address technical comments

1.0 PURPOSE

The purpose of this Standard Operating Procedure (SOP) is to provide the methods used by a Board Certified Histologist for evaluating the histology of small mammal tissues collected at the Libby OU3 and reference area sites.

2.0 LABORATORY EQUIPMENT

- Paraffin
- Microtome
- Stains
- Microscope for tissue examination
- Laboratory notebook
- Indelible ink pen for recording findings.

3.0 METHODS

3.1 Tissue Preparation

Tissues will arrive at Northwest ZooPath in 10% phosphate buffered formalin. Tissues will be trimmed beneath a fume hood, processed routinely and embedded in paraffin (total of 5 blocks per study animal) as described in the steps below. Sample numbers will be recorded and be traceable for each tissue evaluated. Target tissues that will be evaluated include the complete GI tract (esophagus, stomach, small and large intestine), the complete pulmonary tract (trachea, larynx, bronchi and lung lobes), thyroid and adrenal glands. For each animal evaluated these tissues will be embedded and evaluated as follows:

1. Sections of trachea, left mainstem bronchus and representative sections of cranial, middle and caudal portions of left lung (one lobe) will be trimmed in 1 block.

2. Sections of mainstem right bronchus, and representative portions of the middle of each of the four right lung lobes (apical, azygous, cardiac and diaphragmatic, inked for site) will be processed in 1 block.

3. Sections of the esophagus and portions representing all three regions of the stomach will be processed in 1 block.

4. Longitudinal sections of adrenal and thyroid will be processed in 1 block.

5. Sections of duodenum, jejunum, ileum, cecum, and colon will be processed in 1 block.

6. Representative sections through the middle of any abnormal lesions identified during gross necropsy that occur in non-target tissues will be processed in 1 block.

Sections will be trimmed at 5 micron thickness on a microtome, mounted on frosted glass slides and marked with the general lesion location within the tissue and the appropriate Index ID number from the jar containing tissue cassettes. Sections will be stained with hematoxylin and eosin. Additional sections may be stained by other techniques such as Masson's trichrome for collagen, or Prussian blue for iron, depending on histologic findings in the examined tissues. Immunohistochemical staining to differentiate pulmonary adenocarcinoma from mesothelioma (if needed) would be outsourced.

3.2 Tissue Reading and Scoring

Sample number will be recorded for each tissue prior to reading. Slides will be examined microscopically by a board certified veterinary pathologist (Dr. Michael Garner, Northwest ZooPath) and results recorded. The presence and type (e.g., necrosis, fibrosis, inflammation, etc.) of lesions will be recorded for each animal's tissues. The tissues will be tabulated and scored based on lesion severity and microscopic distribution as follows:

Lesion Type	Score Assigned
a. No lesion	0
b. Minimal lesion	1
c. Mild lesion	2
d. Moderate lesion	3
e. Marked lesion	4
f. Severe lesion	5

Representative microphotographs will be taken to illustrate what the pathologist identifies as meeting the lesion types identified in b – f above. The lesion score for each tissue will be multiplied by a pathos factor of either 1 or 2, recorded by the pathologist, to address if the lesion is believed attributable to asbestos (factor of 2) or other (non-asbestos) causes (factor of 1). Lesions that naturally occur in the reference (control) animals will be useful in evaluating and considering attribution to asbestos for animals collected from the asbestos site. Lesions that may be attributed to asbestos, or possibly attributable to asbestos, would include: fibrosis, asbestos or similar crystal deposition, mesothelial cell pathologic changes, mesothelioma, squamous cell carcinoma, bronchogenic carcinoma, polyps / other mucosal proliferative changes of the alimentary tract, proliferative lesions of the thyroid, and any other lesions similar to those that have been convincingly reported in the literature (including NTP studies) as linked to asbestos.

For each animal, the individual tissue scores will be provided as well as a total animal score. The total animal score represents the sum of the individual tissue scores divided by the number of tissues evaluated to obtain an animal-specific numeric "score".

If necessary following discussion with EPA, additional expert opinions will be obtained from pathologists that have special interest, expertise or publications pertaining to the lesions of interest.

3.3 Reporting

A formal report will be generated that contains tabulated data for each animal (total score) and its tissues (lesion scores), including a cover letter summarizing the pathologist's interpretation of the findings. Where necessary, interpretation of histology findings will be referenced by supportive literature as available.

The histology report will be included in its entirety in the Parametrix Small Mammal Data Report that is submitted to Remedium and EPA. The Parametrix report will include details of trapping and necropsy (animal weights, sex, pregnancy status, etc.) as well as photographs that are representative of trapping activities and all animal necropsy photos.

All preserved tissues, and preserved animal carcasses will be maintained under Chain of Custody for five years at the histology lab. After that time, Parametrix will either be notified by the laboratory of a request to dispose of any tissue or arrangements will be made with the laboratory to hold the tissues for a longer period. Slides and blocks are held indefinitely at the histology laboratory unless other arrangements are made with the study investigators

Date: August 18, 2009

SOP MAMMAL-LIBBY-OU3 (Rev. 1)

Title: SMALL MAMMAL COLLECTION AND PROCESSING

APPROVALS:

TEAM MEMBER SIGNATURE/TITLE		DATE
EPA Remedial Project Manager		8/18/09
SOP Author		08/18/09

Revision No.	Date	Reason for Revision
0	05/21/2009	—
1	8/18/09	Address technical comments

1.0 PURPOSE

The purpose of this Standard Operating Procedure (SOP) is to provide a standardized method for collection of small mammals for biological surveys, chemical analysis of tissues, and/or histopathological examination. This procedure will be used by USEPA Region 8 for the Remedial Investigation work for Operable Unit 3 performed at the Libby Asbestos Superfund site.

This document focuses on methods and equipment that are readily available and typically applied in collecting small mammals. It is not intended to provide an all-inclusive discussion of small mammal collection methods. Specific sampling problems may require the adaptation of existing equipment or design of new equipment. Such innovations shall be clearly described in the project-specific sampling plan and approved by the Project Manager and the Quality Manager.

2.0 RESPONSIBILITIES

This section presents a brief definition of field roles, and the responsibilities generally associated with them. This list is not intended to be comprehensive and often additional personnel may be involved. Project team member information shall be included in project-specific plans (e.g., work plan, field sampling plan (FSP), quality assurance plan, etc.), and field personnel shall always consult the appropriate documents to determine project-specific roles and responsibilities. In addition, one person may serve in more than one role on any given project.

Project Manager: Selects site-specific sampling methods, sample locations, and constituents to be analyzed with input from other key project staff.

Quality Control Manager: Overall management and responsibility for quality assurance and quality control (QA/QC). Selects QA/QC procedures for the sampling and analytical methods, performs project audits, and ensures that data quality objectives are fulfilled.

Field Team Leader (FTL) and/or Field Biologist: Implements the sampling program, supervises other sampling personnel, and ensures compliance with SOPs and QA/QC requirements. Prepares daily logs of field activities.

Sampling Technician (or other designated personnel): Assists the FTL, field biologist, or engineer in the implementation of tasks. Performs the actual sample collection, packaging, and documentation (e.g., sample label and log sheet, chain-of-custody record, etc).

3.0 EQUIPMENT

3.1 Organizational and Safety Equipment

- Sampling and Analysis Plan (SAP) and Quality Assurance Project Plan (QAPP)
- Health and Safety Plan (HASP)
- Safety equipment as specified in HASP
- Clipboard, writing utensils, permanent waterproof ink marker
- Site maps
- Global Positioning System (GPS) navigation/survey equipment
- Digital camera

3.2 Trap Setting and Data Recording Equipment

- Data collection sheets and field log books
- Tape measure, 100-foot length
- Survey flags and flagging tape
- Leather gloves
- Backpack/bags to carry traps in field
- Basket or box for trap transfer to laboratory
- Duct tape
- Traps
- Bait (e.g., oats, peanut butter)

3.3 Sample Processing and Shipment Equipment

- Nitrile gloves
- Dissecting equipment (trays, instruments)
- Wet ice
- 10% Buffered formalin
- Portable balance, 400g capacity, 0.1g readability
- Euthanasia chamber with CO_2 source
- Storage coolers
- Small/large re-sealable plastic bags
- Glass and/or plastic vials for tissue samples
- Garbage bags
- Plastic sheets for tables
- Duct tape

3.4 Decontamination Equipment

- 5-gallon plastic buckets
- Hypochlorite bleach or Lysol™ disinfecting solution
- Scrub brushes
- Paper towels
- Garbage bags

4.0 SAMPLE COLLECTION

4.1 Preparation

A scientific collection permit shall be obtained from the appropriate federal or state agency. Additionally, an IACUC (Institution for Animal Care and Use Committee) approval will be obtained from the appropriate State agency. Most states have permit information available on the internet. A natural heritage search for threatened or endangered species should also be requested from the state. In addition, permission from the landowner(s) must be received prior to trapping at the site or reference areas.

4.2 Sampling Location Selection

The exact locations of the sampling areas and placement of trap lines should be made during an initial field reconnaissance based on the identified habitats, terrain, access and other considerations. Areas identified for small mammal trapping are described in the site-specific sampling and analysis plan (SAP).

4.3 Targeted Species

The mammalian species targeted for collection are described in the site-specific SAP. For the purposes of the OU3 Phase III investigation, the target species include the deer mouse (*Peromyscus maniculatus*) and the southern red-backed vole (*Clethrionomys gapperi*).

4.4 Trap Selection

While many types of traps are available for the collection of small mammals, this small mammal collection SOP will use live traps including Sherman Live traps and Haveahart traps. Sherman Live traps are a type of box trap that are the most effective for capturing small terrestrial mammals unharmed (Wilson et al., 1996). As shown in Figure 1, this trap is rectangular in shape with a spring-loaded door that becomes triggered once an animal enters the trap. Box traps are recommended over simple snap traps due to reduced occurrences of predation and trap disturbance by raccoons and deer. Snap traps are lightweight and easily triggered or moved by non-target species. In addition, once an animal is captured in a snap trap, it becomes a likely target for predation. The heavier box trap, with solid sides, is better suited to withstand disruption by predation. Live trapping is also preferred for the collection of samples for histopathology examination. Animals collected from kill traps may decompose prior to collection making tissue examination impossible. The Sherman Live traps come in a variety of sizes. Pearson and Ruggiero (2003) completed small mammal trapping in nine west-central Montana forest stands using Sherman live traps. The most common species they collected in traps were the deer mouse and southern red-backed vole. This indicates that the Sherman live traps can be successful at capturing the target species.

Havahart traps (www.havahart.com) (Figure 1) may also be used. These traps may prove more successful at capturing voles. These traps are not completely enclosed so they allow for examination of the captured organism. However, they are also more prone to predators killing or removing the captured mammals.

4.5 Trap Placement

Methods for capturing mammals and in particular the use of trap arrays are reviewed by Wilson et al. (1996). Typical methods of trap placement include transects, grids and webs. Pearson and Ruggiero (2003) compared transect versus grid trapping arrangements for sampling small mammal communities in two forest cover types in west central Montana. They found that transect arrangements yielded more total captures, more individual captures and more species than grid arrangements in both cover types in both of the years examined. Differences between the two methods were greatest when small mammals were least abundant. Based on this reported efficiency and the lower level of effort required for the line transect method compared to the grid method, the line transect trap method will be used to collect small mammals at Libby OU3.

Traps will be placed along trap lines at spacing intervals appropriate to field conditions, as outlined in the site-specific SAP and the Reconnaissance Memorandum. Transect lines should be numbered or lettered sequentially. Each individual trap along the transect line should also be assigned a number, based on its position along the line, and the GPS coordinates shall be measured and recorded. The location and orientation of each transect line should be sketched in the field logbook and also recorded on either a site map or an aerial photo. The start and end of each grid line or trap line should be marked with a survey flag and/or length of flagging tape tied to a branch at eye level. The flag or flagging should be labeled with the area identifier (e.g., station ID) and transect line identifier using a thick waterproof marker. Each trap should be labeled with the area identifier, the transect line identifier, and a unique trap number using a thick waterproof marker in the following manner.

SM-__- x – y

where:

- SM-__ is the small mammal station ID (S = site; R = reference)
- x is the unique transect line identifier
- y is the unique trap number on the transect line

In heavily vegetated areas, individual trap locations may also be marked with labeled survey flags. This simplifies trap relocation and reduces habitat destruction during subsequent trap checks. Flags should be placed so that they do not impede an animal's progress toward the trap. Traps should be placed at habitat features (e.g., log, tree, runway, burrow) as long as they lie within 2 meters of the point. Traps should seldom be set in open areas, since small mammals usually avoid these areas due to the increased likelihood of predation. Success can still be

increased by placing traps along fallen logs, large roots, or in brushy areas. However, traps should be placed so that the release is not impeded by vegetation or other obstructions.

4.6 Trap Setting and Baiting

Traps will be set at dusk and checked after the first 2 hours of sunlight in order to capture diurnal (active in the daytime), nocturnal, and corpuscular (active at dusk and dawn) animals. Traps will be baited when they are set. Bait should be carried in a re-sealable plastic bag and dispensed as needed. The bait will consist of a mixture of 50:50 peanut butter and rolled oats. [Note: The relative proportions of each can be modified to suit field conditions (e.g., use less peanut butter in warmer weather)]. Traps should be baited so that the bait does not fall off.

4.7 Trap Checks

A field team of at least two people will be used to check the traps, handle and capture the animals, and record the necessary information.

When a sprung trap is located (Sherman or Havahart), it will be carefully picked up and checked for captured animals. If the trap contains a non-target species, the field personnel will record the required information for the animal on the Small Mammal Trapping Log (Appendix A). The animal will then be released in the vicinity of the capture location.

All living individuals of either target species (deer mouse or red-backed vole) will be inspected in the field to evaluate the size. Emphasis will be placed on selecting the oldest (largest) individuals for use, while juveniles and small adults will be released if an adequate number of full-grown adults can be obtained. All animals selected for evaluation will be promptly transported to a pre-established station for necropsy and collection of target tissues for histopathology and potential tissue burden analysis, as described below.

If no animal is discovered inside the trap, the trap will be placed back in the "unarmed" position on the ground until dusk and then reset and re-baited.

All personnel performing trap checks should wear appropriate personal protective equipment as specified in the site-specific Health and Safety Plan (HASP).

4.8 Data Recording for Trapped Mammals

The field team will record the check date and time, the transect line identifier, and trap number for each trap in a Small Mammal Trapping Log (Appendix A). A separate small mammal trapping log sheet will be maintained for each trapping location over the duration of the trapping event.

Unique field sample IDs for each small mammal collected will be generated as follows:

SM-__ - x - y - z

where z is the unique sequential identification number small mammal (e.g., if two small mammals are captured at the same trap on different days, the z identifier for the first mammal will be '01' and the z identifier for the second mammal will be '02'). For example, field sample ID "SM-S-B-03-02" corresponds to the 2nd small mammal collected in trap #3 from transect line B at the Site sampling station.

All target species collected will be euthanized in the field processing laboratory. Animals will be held alive in the traps at the field processing laboratory until they are ready for euthanization and necropsy. Animals will not be held for more than 24 hours post collection and will be provided food (food pellets) and water as necessary to maintain animal health and minimize animal stress. When ready for processing, the animals will be euthanized via carbon dioxide exposure. Carbon dioxide exposure is a humane method of euthanasia approved by the American Veterinary Medical Association (AVMA, 2007) and the Institutional Animal Care and Use Committee (IACUC). Carbon dioxide asphyxiation renders the animal moribund and easier to handle. Cervical dislocation on the moribund animal (after carbon dioxide asphyxiation) will be performed to ensure animals do not recover from the asphyxiation. After euthanization, the animals will be maintained in separate containers labeled with the unique mammal field sample ID number (e.g., SM-S-B-03-02) until they can be examined.

The following information for each trapped mammal will be recorded in the Small Mammal Trapping Log (Appendix A):

Species

The species is recorded by a common name abbreviation. Abbreviations are listed in the following table. It is recognized that some small mammal species (e.g., shrews) will not be readily identifiable to species except through detailed dentition examination (not practical). Where a species is indeterminate only the genus will be recorded on the Small Mammal Trapping Log.

Common Name	Abbreviation
Dusky or Montane Shrew	DSKS
Masked Shrew	MSKS
Pygmy Shrew	PYGS
Vagrant Shrew	VAGS
Northern Flying Squirrel	NFSQ
Red-tailed Chipmunk	RTCM
Bushy-tailed Woodrat	BTWR
Columbian Ground Squirrel	CGSQ
Deer Mouse	DEMO
Golden-mantled Ground Squirrel	GMSQ

Heather Vole	HEAV
Hoary Marmot	HORM
Long-tailed Vole	LTDV
Mountain Cottontail	MTCT
Northern Pocket Gopher	NPGO
Pika	PIKA
Red Squirrel	RESQ
Southern Red-backed Vole	SRBV
Snowshoe Hare	SNSH
Yellow-bellied Marmot	YBMA
Yellow pine chipmunk	YPCM
Western Jumping Mouse	WJMO
Water Shrew	WATS
Water Vole	WATV

General Lifestage

If possible, the general lifestage of the mammal should be recorded on the Small Mammal Log while at the trap site using the designations listed below. In general, guidelines for aging mammals are derived from the findings of field studies that mark individuals at birth and follow them through adulthood. Aging criteria for mammals are generally taxa specific. For the most part, mammals are assigned to broad age classes (following table) relative to developmental or reproductive milestones (Kunz et al., 1996). During the Phase III sampling efforts, it will be difficult to assess absolute age in the field as the methods for aging rely on verifying age-related differences (e.g., body size, ossification of long bones, tooth wear) by measurement on a statistically appropriate number of known-age individuals (Kunz et al., 1996). Mammals captured in the field can be aged by collecting body measurements and evaluating reproductive criteria relative to the broad age categories listed below.

Lifestage	**Description**
Juvenile	A weaned young mammal that still associated with its mother or siblings and may nurse infrequently; usually smaller than a subadult.
Immature	A young mammal that is neither fully grown nor sexually mature.
Subadult	A young mammal that is not fully grown but that may or may not be sexually mature or have adult pelage.
Adult	A fully grown mammal that is sexually mature.
Old adult	An animal that shows extreme tooth wear and/or poor body condition.

Dead Animals

Animals found dead in a trap will not be selected for gross necropsy and the collection of target tissues. Dead mammals will be returned to the collection environment well away from the area of capture to discourage attracting predators.

4.9 Animal Examination and Gross Necropsy

Animals selected for evaluation will undergo external examination to identify animal sex (confirmed through necropsy) and record animal weight prior to the start of the necropsy. This information will be recorded on the Field Sample Data Sheet labeled with the animal field sample identification number.

Necropsy for examination of gross and microscopic lesions in several target organs will then proceed. As specified in the site-specific SAP, the target organs which will be removed following necropsy for histology are (i) complete pulmonary tract (with larynx); (ii) complete gastrointestinal tract; (iii) thyroid; and (iv) adrenals. EPA has identified other tissues of interest for histopathology as well, these are: liver, kidneys, uterus, heart, and ovaries. Decisions regarding the need to conduct histologic examination of these other tissues will be made following evaluation of the weight of the histology evidence regarding the presence of asbestos-related lesions in the identified target organs. Accordingly, only the target tissues will be removed for histology at this time.

Because there is interest in understanding the age of the small mammals collected and age can only be accurately established post mortem, the eyeballs of each small mammal will be removed from each animal for subsequent lens extraction, drying and weighing to determine animal age.

Animals selected for necropsy will be euthanized and placed (temporarily) in a baggie marked with the unique small mammal field sample ID (e.g., SM-S-B-03-02). The animal will then be lightly wetted for the gross necropsy examination and collection of tissues.

When handling animals for necropsy, the primary consideration should be personal safety. Field personnel should be trained in techniques to handle mammals in a manner to minimize potential transfer of wildlife diseases. The necropsies will be performed by experienced and trained personnel.

Each necropsy technician will have a necropsy log book to record data and observations. The general steps for necropsy include:

- Record animal field sample ID number in necropsy logbook.

- Examine external body surface (through fur) for lesions (open or closed), tumors etc.. Record location(s) and descriptions of any lesions / abnormalities in the necropsy logbook with other general observations.

- Photograph both the dorsal and ventral views of the animal using appropriate scale and with animal field sample ID number clearly visible.

- Once the body cavity is fully opened, a photograph (with animal ID number visible to the extent practical) will be taken to show the orientation, color and gross appearance of internal organs.

- Examine the internal organs for color, size (swelling), and other gross abnormalities, including the presence of macroscopic lesions, nodules or plaques.

- Suspect lesions (i.e., not obviously parasite related) should be recorded using photographs (animal ID number must be made visible in some manner in each photo).

All observations, including number of photographs (and frame numbers) taken during the gross necropsy should be recorded in the necropsy log book.

4.10 Collection and Preparation of Tissue Samples

Selected tissues will be removed and portions of the tissues will be sent for histopathology examination and, if sufficient mass (> 20 mg) is available, for possible future analysis of asbestos levels by Transmission Electron Microscopy (TEM). Target tissues are identified in Section 4.9 of this SOP and also specified in the site-specific SAP..

For each tissue collected, one half or representative portions will be preserved for histopathological examination. When sufficient tissue mass is available, a second half or representative section will be removed and kept frozen for possible future tissue asbestos analyses. A Small Mammal Tissue Field Sample Data Sheet (FSDS) (provided in OU3 SOP No. 9) will be completed for each small mammal from which tissues are collected for potential tissue burden analysis. If a tissue is too small to provide a section to the analytical laboratory for future tissue asbestos analysis, histology results will dictate whether paraffin embedded tissues in the histologist's possession will be submitted instead.

For all histology tissue samples collected from a given animal, the sample will be placed into a tissue cassette marked (sharpie) with tissue type (small intestine, etc.) and the cassettes placed into a single plastic screw top container in a volume of 10% buffered formalin solution sufficient to ensure adequate preservation of the contents in all of the cassettes. The remainder of the animal carcass will be placed in a second plastic screw top sample container containing sufficient 10% buffered formalin solution to ensure adequate preservation. Plastic containers will each be labeled with the animal identification number.

For each sample collected for potential future asbestos tissue burden analysis, the sample will be placed in a pre-numbered and pre-weighed (by EMSL) glass scintillation vial. The vial will contain no fluid. The vial number, the field ID number for the source animal, and the tissue type placed into each numbered vial will all be recorded on the FSDS. All vials with tissue samples will be maintained on wet ice until delivered to EMSL in Libby, MT for final weighing and storage for potential future tissue analysis.

Personnel should wear powder-free gloves for labeling and handling of sample containers, and for the dissection procedure. Plastic bottles used for preserving (with 10% neutral buffered formalin) tissue specimens should have a wide mouth and threaded caps for secure closure. Plastic bottles eliminate potential breakage problems. All wide-mouth plastic collection bottles need to be labeled before collecting tissue samples. Sample labeling procedures are specified in OU3 SOP No. 9.

Dissecting tools will be dedicated to specific procedures. Dissecting tools used to expose the internal organs will not be used to remove tissues. Dissection tools should be decontaminated between each animal examined.

Variability between species may result in some differences in the appearance and relative size of particular organs and tissues, but their location will be similar among species. When dissecting tissues, care should be taken to avoid squeezing or distorting tissues with forceps.

Procedures for target tissue sample removal and preparation are described below:

- Larynx. The larynx will be examined in place on the trachea and will be submitted intact (with the trachea) for histological examination. The larynx is too small for a second tissue split for tissue burden.

- Thyroid. The thyroid will be examined in place on the trachea and will be submitted intact (with the trachea) for histological examination. The thyroid is too small for a second tissue split for tissue burden.

- Gastrointestinal (GI) Tract. Gently remove the entire GI area. Examine the tract carefully for lesions that are not parasite-related. The GI tract should be divided into four sections: esophagus, stomach, small intestine, and large intestine. For each GI section, the samples will be divided for tissue burden analysis and histology as follows:

  -Asbestos tissue burden: pieces of each of the four GI section will be placed in individual, pre-numbered glass scintillation vials. The tissue type placed in the vial and the vial number will be recorded on the FSDS. All vials for a given animal will be placed in a ziplock baggie labeled with animal ID number and maintained on wet ice until delivered to EMSL in Libby.

-Histology: the remaining (and largest) parts of each of the four GI sections will be placed in individual tissue cassettes marked (Sharpie) with tissue type and the four cassettes placed in a labeled (with animal sample identification number) plastic jar containing adequate 10% buffered formalin to ensure preservation of all cassette contents.

- Lungs. Remove the entire pulmonary tract consisting of the trachea with bronchi and lungs; the thyroid and larynx will be intact and attached to the trachea. Examine carefully for gross lesions or abnormalities. For tissue burden, remove a small section of each of the lung lobes (four in right lung, 1 in left lung) and place these pieces in a numbered glass scintillation vial. Circle the tissue type and record the scintillation vial number on the FSDS. Using a 6 cc syringe then perfuse the lungs with 10% neutral buffered formalin. This tissue should not be placed in a tissue cassette at the request of the histologist and instead allowed to free float in the plastic jar with the other tissue cassettes.

- Adrenals. Both adrenals will be submitted for histological examination as each is likely too small to meet the minimum tissue weight needed for asbestos tissue burden. Remove, examine carefully, and place in a marked tissue cassette. The cassette will be placed in the same labeled formalin-filled plastic jar containing other animal target tissues for histology.

- Gross Lesions. If any non-parasite related lesions are observed, on non-target tissues during the examination, collect separate tissue samples for microscopic examination and other analyses. Cut a thin (1/8" – 1/4") section of tissue that includes all or portions of the lesion and adjacent apparently healthy tissue. Use caution not to crush tissue in or around the lesion. Place the lesion tissue sample in a tissue cassette (one per cassette) marked with tissue type (liver, etc.) and "lesion section". Place the cassette with the lesion section in to the same formalin-filled jar containing the other target tissues for histology. Unless a lesion is generally large, it will not be practical to remove parts of it for asbestos analysis. As noted previously, should a tissue be too small to provide to the analytical laboratory for future tissue asbestos analysis, histology results will dictate whether paraffin embedded tissues in the histologist's possession should be submitted.

- Eye Lens. The eyeballs of each animal will be removed and preserved in a volume of 10% formalin. Gently remove the intact eyeballs and place in a pre-labeled (with animal ID number) plastic screw top jar prefilled with sufficient 10% neutral buffered formalin to ensure preservation. Eye lens dry weight will be determined in the future using a modification of Lord's technique (Lord, 1959). The fixed lenses are removed from preserved eye balls within 12 months of preservation and dried at 95 degrees C until they reach a constant weight, usually in about 96 hours. Lenses are then removed from the oven and weighed to the nearest 0.2 mg on a precision balance.

- Carcass. What remains of the animal after all target tissue samples have been removed (the carcass with fur on) will be placed in a separate pre-labeled (with animal ID number) plastic screw top jar prefilled with sufficient 10% neutral buffered formalin to ensure adequate preservation. The carcass sample will be archived at the histology lab for possible future use.

Though pre-filled formalin jars with screw tops will be used, formalin is classified as hazardous and the necropsy team should take appropriate measures to prevent skin contact or vapor inhalation (i.e., keep jars containing formalin tightly closed and use respiratory protection).

Twice per day a piece of control tissue (liver or other store bought tissue) will be placed in a numbered glass scintillation vial to act as a tissue blank. The vial number will be recorded on the FSDS sheet and the tissue identified on the FSDS as a tissue blank. Tissue blanks should be collected once at mid-day and again near end of day and submitted with other tissues to the approved EPA laboratory for possible asbestos analysis.

4.11 Sample Packaging, Handling and Transportation

Field personnel will complete a chain-of-custody form for collected tissue samples in accord with Libby OU3 *Sample Documentation* (SOP No. 9) and *Sample Handling and Shipping* (SOP No. 8) procedures. It is the responsibility of the Parametrix FLT and QA Field Officer to ensure that the containers are labeled and properly sealed to prevent leakage during transport. Pack the containers for shipping to minimize jarring the containers during shipment. Note that exposure of histological samples to extreme temperatures during shipping should be avoided since they can alter tissue characteristics, making tissues unsuitable for histological examination. Check with local couriers regarding current requirements or restrictions for shipment of formalin.

Samples collected and preserved for histopathology shall be transported to a laboratory qualified and experienced in performing histopathology examination of tissues. The histopathology laboratory will be responsible for further fixation and preparation of samples for histopathological examination in accordance with the Histology SOP (SOP# HISTO-LIBBY-OU3 (Rev. 1). Samples collected for asbestos residue analyses will be transported on wet ice to an EPA-approved analytical laboratory (EMSL, Libby, MT).

5.0 DATA VALIDATION

All tissues collected for histology and tissue burden analysis will be recorded on Chain of Custody (COC) forms appropriate to the delivery location of the tissue. Tissues for histology and carcasses for archiving will both be shipped in separate and well packed coolers to Northwest ZooPath. Tissues for possible future asbestos burden will be delivered by hand under COC to EMSL in Libby, MT. Eye tissues for later age determinations will be delivered under COC to Parametrix' laboratory in Albany, OR.

All data recorded on trapping logs and FSDS forms will be checked by the FTL against records kept in field logbooks. It is also the responsibility of the FTL to verify the contents of each shipping cooler against the chain-of-custody form prior to shipment.

6.0 DECONTAMINATION AND HEALTH AND SAFETY

According to the Centers for Disease Control and Prevention (CDCP), several species of small mammals (e.g., *Peromyscus maniculatus, Sigmodon hispidus,* and *Microtus pennsylvanicus*) have been found to carry and potentially transmit a hantavirus to humans (CDCP 1996). Field biologists and other personnel who are exposed to small mammal body fluids and excreta are particularly at risk of Hantavirus infection (Mills et al. 1995). This virus can cause hantavirus pulmonary syndrome (HPS), which has been fatal to a high percentage of exposed individuals. Individuals who plan to trap, handle, process, or otherwise be involved in any activities related to small mammals should be educated about the inherent risks of such activities, as well as ways to minimize those risks.

All equipment used in the sampling process shall be decontaminated prior to field use and between sample locations. Decontamination procedures are presented in OU3 SOP No. 7. Personnel shall don appropriate personal protective equipment as specified in the health and safety plan. Investigation-derived waste, if any, generated in the sampling process shall be managed in accordance with the procedures outlined in OU3 SOP No. 12.

7.0 REFERENCES

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FIGURES

Figure 1. Examples of Small Mammal Traps

Sherman Live Trap

Havahart Trap

APPENDIX A
SMALL MAMMAL TRAPPING LOG

LIBBY OU3: Small Mammal Trapping Log (Rev 1)

Station ID:_____ **Field Logbook ID:**_____ **Logbook Page No.:**_____

Collection Date/Time [mm/dd/yy hh:mm]	**Transect ID**	**Trap #**	**Animal #** [see Note 1]	**Genus/Species** [see Note 2]	**Lifestage** (circle one)	**Captured Alive (A) Dead (D)**	**Notes of Field-Observed Physical Abnormalities (if any)**
					JV IM SA AD OA		
					JV IM SA AD OA		
					JV IM SA AD OA		
					JV IM SA AD OA		
					JV IM SA AD OA		
					JV IM SA AD OA		
					JV IM SA AD OA		
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					JV IM SA AD OA		
					JV IM SA AD OA		
					JV IM SA AD OA		
					JV IM SA AD OA		

Notes:

Age Categories: JV = juvenile; IM = immature; SA = sub-adult; AD = adult; OA = old adult

[1] If multiple animals are collected from the same trap, they should be assigned unique sequential identifiers.

[2] See SOP MAMMAL-LIBBY-OU3 for species identifier codes; some mammals may not be identifiable to species (record genus).

FSDS Rev. 1

Sheet No.: SM- _____

LIBBY OU3 FIELD SAMPLE DATA SHEET (FSDS)
SMALL MAMMAL TISSUE COLLECTION FOR TEM ANALYSIS

Field Logbook ID: _____ **Logbook Page No.**: _____

Necropsy Date: _____ **Personnel Initials**: _____

Small Mammal Field ID: SM–_____–_____–_____–_____

*[**SM** - station ID - transect ID - trap# - animal#]*

Animal Weight (grams): _____(initial) _____(w/o uterus if pregnant) **Sex (circle one):** M F UNK

	TISSUE #1	TISSUE #2	TISSUE #3	TISSUE #4
Tissue Type (circle one):	ESO STO SMI LGI LNG Other:_____	ESO STO SMI LGI LNG Other:_____	ESO STO SMI LGI LNG Other:_____	ESO STO SMI LGI LNG Other:_____
Vial No.:				
Field QC Type (circle one):	FS FD TB	FS FD TB	FS FD TB	FS FD TB

	TISSUE #5	TISSUE #6	TISSUE #7	TISSUE #8
Tissue Type (circle one):	ESO STO SMI LGI LNG Other:_____	ESO STO SMI LGI LNG Other:_____	ESO STO SMI LGI LNG Other:_____	ESO STO SMI LGI LNG Other:_____
Vial No.:				
Field QC Type (circle one):	FS FD TB	FS FD TB	FS FD TB	FS FD TB

	TISSUE #9	TISSUE #10	TISSUE #11	TISSUE #12
Tissue Type (circle one):	ESO STO SMI LGI LNG Other:_____	ESO STO SMI LGI LNG Other:_____	ESO STO SMI LGI LNG Other:_____	ESO STO SMI LGI LNG Other:_____
Vial No.:				
Field QC Type (circle one):	FS FD TB	FS FD TB	FS FD TB	FS FD TB

Sex Descriptors: M = male; F = female; UNK = unknown (cannot determine sex)
Tissue Type Descriptors: ESO = esophagus; STO = stomach; SMI = small intestine; LGI = large intestine; LNG = lung
Field QC Type Descriptors: FS = Field Sample; FD = Field Duplicate; TB = Tissue Blank

Comments: _____

For Data Entry Completion (Provide Initials)	Completed by	QC by

4.2.5 Exposure of Mammals to Asbestos

4.2.5.1 Strategy of the Phase III Investigation

EPA considered several alternative strategies for an investigation of risks to mammals in OU3.

First, EPA considered whether it was necessary to characterize risk at multiple locations in OU3 (selected to include a range of measured LA concentration levels) to support risk management decisions, or whether collection of data from only two locations (area of highest measured LA concentrations vs. reference) would be sufficient. EPA determined that collection of data from only two locations (area of highest measured LA concentrations vs. reference) was the most appropriate first step. If no ecologically significant effects on mammals are observed in animals from the area of highest measured LA concentrations, then additional field investigations of mammals are unlikely to be needed. If ecologically significant effects are observed, then additional studies at multiple locations may be needed to establish either an exposure response relationship, or to derive an empiric map of the extent of the impact.

Next, EPA considered where the study area should be located. Three locations were considered: a) on the mined area, b) in the forest area surrounding the mine, and c) along streams and ponds. EPA determined that a study of risks to mammals from LA along streams and ponds was not a high priority because risk management decisions regarding ecological risks from LA in surface water and sediments along streams and ponds are likely to be determined mainly by risks to aquatic receptors, rather than to mammals. Similarly, a study in the mined area was not considered to be high priority because the mined area is heavily disturbed and the habitat for small mammals is substantially altered. Although a colony of Columbia ground squirrels (*Spermophilus columbianus*) have been observed in the disturbed mine area, the area occupied by these receptors represents only a small portion of the mined area, so a study of these receptors would have only limited utility in decision-making. In contrast, the forested area impacted by releases of LA is substantially larger than the mined area and habitat is not altered by mining. The habitat is suitable for a wide range of mammalian receptors. Based on these considerations, EPA determined that a study in the forested area would be most useful for risk-management decision making. Such a study will help answer the question of whether response actions need to be developed and evaluated to address unacceptable risks to mammals within the forested area. A final decision regarding the potential need for an evaluation of risks to mammals within the mined area will be deferred until the results for small mammals are available from the forested area.

4.2.5.2 Data That Are Valuable for Evaluating Effects of LA on Mammals

As discussed in the Problem Formulation (EPA 2008d), a weight of evidence approach will be used to evaluate ecological risks within OU3. One potential line of evidence used for mammals is the computational hazard quotient (HQ) approach. This approach requires a) accurate and representative measures of exposure (dose) of ecological receptors to site media, and b) a reliable dose-response relationship for an ecologically relevant response (a decrease in growth, reproduction and/or survival). However, in the case of LA, neither of these two types of data is presently available for mammals. Because of this, other lines of evidence will be considered to evaluate potential risks to mammals from LA in OU3. The other lines of investigation under consideration are laboratory-based oral and inhalation toxicity studies of LA in mammals, site-specific population studies, and measurements of *in-situ* exposure and effect.

The Phase III data collection program is focused on measurements of *in-situ* effects and possibly exposure. The goal is to determine if individual mammals from the LA-contaminated forested area have higher incidence and severity of histological lesions and/or gross deformities than mammals from a reference area. If needed to determine whether observed effects are related to exposure to LA, *in-situ* exposures (tissue burdens of LA) may be evaluated if the weight of the histology evidence regarding the presence of asbestos-related lesions in the identified target organs indicates this is necessary.

4.2.5.3 Summary of Existing Data

There are no existing data on *in-situ* measures of either effects (histological lesions) or exposure (tissue burden of asbestos) in mammals at OU3.

4.2.5.4 Data Quality Objectives for Small Mammals

Step 1: State the Problem

Mining operations at OU3 have resulted in the release of LA to the forested area surrounding the mine site, impacting soils, tree bark, and duff. Mammals in the forest area may be exposed to asbestos from contact with these media (mainly soil and duff) both via inhalation and ingestion. However, it is not known if exposures to LA in these media cause unacceptable asbestos related lesions in small mammals when compared to a reference location. The problem to be resolved is: Do the concentrations of LA at the Libby OU3 site cause unacceptable asbestos-related histologic effects in small mammals when compared to a reference location?

Step 2: Identify the Goals of the Study

The Phase III investigation is a focused investigation of effects in one area of the

surrounding forested area that, based on measured concentrations of LA in duff, is maximally contaminated, and to compare the results from this area to an appropriate reference area. Because the area selected would capture maximal exposure levels, if no adverse effects (related to assessment endpoints) are observed in comparison to reference, then further investigations would not be needed (i.e., exposures within areas with lower levels of LA would be less than those in the highest impacted area). If adverse effects (related to assessment endpoints) are observed, then a follow-up study to determine an LA concentration protective of small mammals and an area of concern (spatial) might be required to support risk management decisions.

A secondary goal (conditional on the finding that potentially significant histological effects are occurring in animals from the contaminated area) is to confirm LA exposure in the animals from the contaminated area (by measuring LA in tissues) compared to the reference area. This information will be collected if needed to help determine if the observed effects are attributable to LA exposure.

Step 3: Identify the Types of Data Needed

The data needed to support the primary study goal are quantitative measures of the frequency and/or severity of histological lesions in mammals collected from an area of OU3 that is contaminated with highest levels of LA in duff in the forested area surrounding the mined area and from a reference location where LA contamination is either zero or negligible.

The data needed to support the secondary goal are reliable measures of the LA in tissues in which the histological effects are observed.

Step 4: Define the Boundaries of the Study

Spatial Bounds

In order to maximize the probability of detecting *in-situ* effects if they are present (and minimize the chance of a false negative), it is necessary to collect mammals at a location where exposures to asbestos are expected to be highest. If *in-situ* effects are not observed in the area of highest LA contamination then it is unlikely that effects will be measured in areas of lower LA contamination. Figure 3-4 summarizes the available data on the levels of LA in forest duff, soil and tree bark at OU3. As shown, the highest levels of LA are observed in the area just north (downwind) of the mined area). Based on the duff data, the collection of mammals will occur within a polygon bounded by four sampling locations where the highest LA concentrations have been measured in duff. The four sampling locations and their corresponding LA concentrations in duff are:

Station	Duff (% LA)
SL-15-02	3.65%
SL-45-02	1.74%
SL-45-03	4.27%
SL-75-03	3.52%

This set of 4 stations bounds a polygon that is roughly triangular in shape and covers an area of about 716,000 m^2 (72 Ha).

The reference area should be matched as closely as possible to the habitat of the forested area north of the mined area, but must be located cross-wind or upwind of the mined area, and far enough from the mined area (e.g., > 5 miles) that contamination with LA is zero or negligible. This distance will also ensure that mammals collected at the reference will represent a separate local population from that sampled north of the mine. The reference trapping area should be similar in size as the trapping area north of the site (about 72 Ha). The exact location will be selected during an initial field reconnaissance and will be subject to approval by EPA.

Temporal Bounds

The asbestos contamination of forest soils and duff is not expected to vary with time. However, the level of exposure of mammalian receptors to environmental media is expected to vary over time. For example, weather may influence the releaseability of LA from duff into the breathing zone of mammals, and activity patterns may vary over seasons. Based on these considerations, the Phase III sampling of mammals should occur in late summer (August or September, no later than September 15) when fiber release potential is likely to be high due to dry weather and when small mammals populations are at peak levels.

Target Species

There are many different species of mammalian receptors that may be exposed to LA in OU3, but it is neither feasible nor necessary to attempt to collect organisms from each species. Rather, attention will be focused on species most likely to be maximally exposed to asbestos in soils and forest duff. As part of the Problem Formulation (EPA 2008d) selection criteria were specified and used to identify the species most likely to be maximally exposed to asbestos in forest duff. It is expected that the most exposed species are non-transitory, have a small home range, forage on the ground, burrow into the ground or create shallow runs under forest litter, and have a small body weight. Taking these criteria into account, ground foraging mammals were identified as the mammalian receptor group most likely to be exposed to asbestos. Of the ground foraging mammals

identified within Lincoln County, Montana, the most common species reported are the deer mouse (*Peromyscus maniculatus*) and the southern red-backed vole (*Clethrionomys gapperi*). These two species are identified as the target species.

Results for these two target species will be utilized to evaluate the potential risks to all ground-dwelling mammals, and may also be used to estimate potential effects on other mammalian species that may be exposed in OU3 (taking differences in exposure patterns and feeding behaviors into account).

Target Tissues for Histopathology Examination

Attachment D provides a summary of studies that have been performed in laboratory rodents to identify the effects of inhalation and oral exposure to various types of asbestos (but not LA). The following provides a summary of the data reviewed in Attachment D:

- For inhalation exposures to asbestos (amosite, chrysotile, crocidolite, or anthophyllite) eighteen chronic studies were reviewed. There are no studies available for exposures to LA. With one exception, the only tissues examined in these studies were the lung and mesothelium. One study examined the gastrointestinal tract.

- Following inhalation exposure (at doses where effects were observed) the histological lesions include a) pleural and interstitial lung fibrosis, b) lung cancer (adenomas, adenocarcinomas, or squamous cell carcinomas), and c) pleural and peritoneal mesothelioma.

For oral exposures to asbestos (amosite, chrysotile, tremolite, or crocidolite) eleven chronic studies were reviewed. Of these, five are National Toxicology Program (NTP) studies that examined several tissues including gastrointestinal tract, nervous system, endocrine system, reproductive organs, respiration system, heart, liver and kidneys.

Following oral exposure, there is generally little or no evidence of histological or clinical injury to any systemic tissues, with the possible exception of effects on the gastrointestinal tract. For example, a series of lifetime feeding studies in rats and hamsters did not observe any systemic lesions except for benign adenomatous intestinal polyps in the large intestines of male rats. Studies by other researchers have reported signs of injury to the colon including inflammation, benign productive peritonitis, increases in aberrant crypt foci (putative precursors of colon cancer), and colon cancer (carcinomas, adenomas and adenocarcinomas).

Based on these findings in laboratory animals, it is expected that the primary target tissues of inhalation and oral exposure of rodents to asbestos are the pulmonary tract (including the larnyx, trachea and bronchi) and the gastrointestinal tract (including esophagus,

stomach, small intestine and large intestine). Other possible target tissues are those where pathologic changes were noted but were determined not to be of biological importance in the laboratory study because 1) a similar incidence of pathology was observed in temporal and/or pooled controls or 2) lesions were not observed in target organs. In the studies reviewed, these types of observations were made for effects on the thyroid and adrenals. EPA believes it is appropriate to include these tissues as well as the primary target tissues (pulmonary tract and gastrointestinal tract) in the tissues examined for potential effects in the field collected small mammals. The list of target tissues for collection from the field collected mammals (deer mouse and red-backed vole) includes the following:

- Complete pulmonary tract
- Complete gastrointestinal tract
- Thyroid
- Adrenals.

EPA has identified other tissues of potential interest for histopathology as well, including the kidney, heart, liver, uterus, and ovaries. However, decisions on conducting histological examination of these other tissues will be made by EPA following evaluation of the weight of the histology evidence on the presence of asbestos lesions in the target tissues listed above. If it is determined by EPA that other tissues of potential interest require examination histologically, the archived animal carcass will be readily accessible for tissue extraction and examination.

After collection of the target tissues, the remaining individual organism will be preserved in the event that the histologic findings or other future concerns suggest the need to examine other tissues in the future.

Step 5: Develop the Analytical Approach

The analytical approach is to compare the nature, frequency, and/or severity of histopathological lesions in animals collected from the LA-contaminated study area with that for animals from the reference area. Possible outcomes of this analysis are listed below.

- Outcome 1: There are no statistically significant differences in histopathological effects, and there are no effects that are definitively LA-related (even if not statistically significant). In this case, it will be concluded that adverse effects of LA on mammals are either absent or minimal in OU3, and that no further investigation is needed.

- Outcome 2: Statistically significant differences are observed, and/or effects are observed that are definitively caused by LA (even if not statistically significant). In this event, the nature and severity of the effects will be evaluated to determine if

the effects are likely to result in an impact on growth, reproduction or survival of the individual. If so, then further investigation may be needed to determine: a) if LA is the cause of the lesions (e.g., by measuring LA tissue burdens in the exposed animals), b) whether the effects result in an ecologically significant effect on the population, and if so, c) to characterize the spatial extent of ecologically significant impacts as may be necessary to support risk management decisions.

Step 6: Specify Performance or Acceptance Criteria

When comparing two data sets (site *vs.* reference), two types of decision errors are possible:

- A false negative decision error occurs when it is decided that there are no important differences between site and reference, when significant differences actually do exist
- A false positive decision error occurs when it is decided that important differences do exist between site and reference, when no significant differences actually exist

As discussed in EPA (2002), the probability of decision errors when comparing two data sets (site *vs.* reference) is controlled by the selection of the null hypothesis, and by selection of an appropriate statistical method to test the null hypothesis. Two alternative forms of null hypothesis are possible:

- Form 1: The null hypothesis is that no difference exists between site and reference. A confidence level of $100(1-\alpha)\%$ is required before the null hypothesis is rejected and it can be declared that the site data are higher than the reference data.

- Form 2: The null hypothesis is that the site is higher than reference by some amount (S) that is considered to be biologically significant. A confidence level of $100(1-\alpha)\%$ is required before the null hypothesis is rejected and it is declared that that the difference between site and reference, if any, is smaller than S.

For the purpose of this effort, the Form 1 null hypothesis is selected for use because it is the most familiar, is the easiest to interpret, and does not require specification of an effect that is presumed to be significant. In accord with EPA (2002), when the Form 1 null hypothesis is used, it is appropriate to select a value of α that is somewhat higher than the usual value of 0.05, such that marginal differences between site and reference are more easily identified as being significant. In accord with this, α is set to 0.20.

Step 7: Develop the Plan for Obtaining Data

Statistical Test

The statistical test that is most appropriate for comparing histological lesions and tissue burdens (if needed) in animals from the site with animals from the reference area can not be determined with certainty until the data are obtained. However, for the purpose of designing the sample collection program, it is assumed that the most appropriate method for dichotomous endpoints (e.g., each tissue from each animal is classified either having or not having a particular lesion) will be the Fisher Exact Test. For continuous endpoints (e.g., histopathological scores are assigned to each tissue of each animal evaluated, as well as a combined score), it is assumed that most appropriate test will be the Wilcoxon Rank Sum (WRS) test (EPA 2002). This is a non-parametric test that is well-suited for comparison of data sets from a site and a reference area. This test would also be well-suited to a comparison of tissue burden data, if needed.

Because it is expected that a histopathological score will be generated for each tissue and/or each animal that will reflect the lesions observed, stratified by tissue type, the severity of each lesion, the pathogenesis of the lesion, and significance, it is expected that the WRS test will be the primary test used in data analysis.

Number of Individuals to be Collected

The power of the WRS test to identify a difference between the site and the reference area depends on the number of observations (i.e., number of animals) in each data set and the variability between the observations. Figure 4-8 shows Test Performance Plots (EPA 2002) that indicate the probability that a statistically significant difference ($p < 0.20$) will be detected between the site and the reference area as a function of the number of animals collected in each data set, the degree of variability between animals within each data set (as reflected in the coefficient of variation, or CV), and the magnitude of the difference between site and reference. As shown, if between-animal variability is low (CV = 0.1, Panel A), then a difference of 20% between site and reference can easily be recognized by collection of as few as 5 animals per area. However, if variability is higher (e.g., CV = 0.6, Panel C), then it would be necessary to collect about 30 animals per area in order to have a high probability (> 90%) of detecting even a 50% difference. Increasing animal number to 50 would offer only a small increase in power to detect a 50% difference, but would not be enough to allow reliable detection of a 20% difference.

At present, no data are available on the degree of variability in histopathological score between animals within an area, or on the potential magnitude of difference between animals from site and reference areas. In the absence of data, it's assumed that the variability in histopathological score between animals within an area is high since exposures are likely to be quite variable. Given this assumption, the target number of

animals per area is selected to be 30. Unless the CV is substantially greater than 0.6, this should provide sufficient power to detect a difference of 50% or less with a probability of about 90% or more using the WRS test. Based on this, the goal is to collect 30 individuals for each of the two target species (deer mouse, red-backed vole) in each area. The total number of individual mammals to be collected is 120.

At present, it is not known whether gender is an important factor that influences the level of exposure or effect. In the absence of information, it is assumed that between-gender variation is not likely to be substantial, and that the data from males and females can be combined into one data set. Therefore, to ensure representativeness, the goal is to collect 15 males and 15 females of each species in each area. If important differences are detected between gender and it is appropriate to stratify the data on this basis, the power of the test to detect differences may be decreased, and additional study might be needed.

To the extent possible, individuals selected for histopathological evaluation should include only adults, with a preference for the largest (heaviest) individuals. This will help ensure that the individuals studied have been exposed for a maximal period of time.

4.2.5.5 Detailed Sampling Design

Initial Field Reconnaissance

Prior to the small mammal trapping effort, an initial field reconnaissance will be completed to map the bounds of the on-site sampling location, to select and map the bounds of the reference area, and to establish trap locations in each area. Key features of the small mammal trapping are discussed in the following sections. The results of the field reconnaissance will be detailed in a report that will be submitted to EPA and MDEQ for review and will be subject to EPA approval. The field reconnaissance report will provide additional details concerning the small mammal trapping program to be performed including the number, arrangement and spacing of traps.

Trap Type

Small mammal collection at Libby OU3 will use a mixture of Sherman Live traps and Havahart traps. Both trap types are effective for capturing unharmed small terrestrial mammals (Jones et al. 1996). Live trapping is selected for the Phase III investigation to ensure that captured animals are suitable for gross and histological examination, since animals collected from kill traps begin to decompose quickly, making tissue examination impossible.

Number, Arrangement, and Spacing of Traps

Although the exact number and arrangement of traps will be detailed in the final field

reconnaissance report, as a guide each sampling area (site, reference) should be trapped using at least 100 traps. Traps should be arranged to provide good spatial coverage across accessible locations within the trapping area, using trap lines. Traps will be spaced at an interval appropriate to field terrain conditions but no closer than 15 feet apart. Exact trap locations may be adjusted based on consideration of the habitat in each trap location..

Trapping Effort

Traps will be set in the evening at dusk and collected in the early morning. The trapping will continue until the target number of organisms is obtained. If the target number of animals for each target species cannot be obtained after sampling over a period of 5 days, then EPA will be contacted to discuss potential changes in the sampling design.

Measurements on Mammals Collected in Traps

For traps that are found to contain a small mammal of any type, the species will be recorded on the small mammal log sheet. All dead animals will be returned to the environment. All living individuals that are not target species (deer mouse or red-backed vole) shall be promptly released.

All living individuals of either target species (deer mouse or red-backed vole) will be inspected in the field to evaluate the size. Emphasis will be placed on selecting the oldest (largest) individuals for use, while juveniles and small adults will be released if an adequate number of full-grown adults can be obtained. All animals selected for evaluation will be promptly transported to a pre-established necropsy and tissue preparation station for necropsy and collection of target tissues for histopathology and potential tissue burden analysis, as described below.

Gross Necropsy and Collection of Target Tissues

Adult animals will be sacrificed for the examination of gross pathology and the collection of target tissues (described previously) for histopathology examination. The details of the examination and collection of tissues is described in SOP MAMMAL-LIBBY-OU3.

Each of the target species collected will be sacrificed by carbon dioxide asphyxiation followed by cervical dislocation. The initial weight of each animal will be obtained and recorded. A gross necropsy will then be performed. The body surface of each animal will be examined and denoted as normal or abnormal with any abnormalities recorded. This includes the location and type of any visible lesions.

Once gross necropsy is completed, the animal will be wetted with a slightly soapy solution to control release of fur into the open body cavity as well as to control airborne release of

any particles/fibers from the animal's fur. Dissection will then be performed to examine internal organs and to obtain tissue samples. The internal organs will be examined for color, size (swelling), and other gross abnormalities including the presence of macroscopic lesions, nodules or plaques. Weights of pregnant females will be recorded with and without the uterus. Photographs will be made to document each examination and each gross abnormality.

From each mammal, a sample of tissue from each target organ will be collected and preserved by placement into formalin fixative for histologic examination. The eyeball from both eyes of each mammal will be removed and preserved in formalin fixative for subsequent analyses of eye lens weight for animal aging. Carcasses will be retained and formalin preserved for archival at the histology lab in the event future analyses of the remaining tissues are needed. The details of the necropsy and collection of target tissues is detailed in SOP MAMMAL-LIBBY-OU3.

When there is sufficient tissue mass, a second sample of tissue will be removed and placed into a glass vial for potential tissue burden analysis. The target mass is 20-200 mg. If a mass of at least 10 mg can not be obtained, then no tissue sample will be collected for potential tissue burden analysis. The details of this procedure are also specified in SOP MAMMAL-LIBBY-OU3.

4.2.5.6 Analytical Requirements

Measurement of Histopathological Effects

The tissue samples will be examined by a qualified pathologist and all findings included in the final small mammal report. The procedures for the examination are detailed in SOP HISTO-LIBBY-OU3.

Measurements of Asbestos Tissue Burden

If the frequency and/or severity of a particular type of lesion is increased in animals from the study area compared to the reference area, but the cause of the increase (LA vs. other factors) is uncertain, then it may be necessary to measure the level of LA in the tissue of interest to help determine if exposure to LA plays a causal role. The exact design of such a study can not be specified *a priori*, but might involve the measurement LA in the tissue of animals a) from the study area with the lesion, b) from the study area without the lesions, and/or c) from the reference area.

If such analyses of LA tissue burden are deemed to be necessary, they will be performed TEM ISO 10312 Method Modification 2, *Analysis of Tissue Samples for Asbestos by TEM* (see Attachment B). In brief, a portion of the tissue sample collected and preserved for histopathological examination will be removed and weighted (wet weight), and then

dried (lyophilized) and ashed at low temperature (plasma ashing). The ashed residue will be resuspended in acid and water and an aliquot deposited on a filter for analysis by TEM. Results will be expressed as fibers of LA per gram (wet weight) of tissue. The target analytical sensitivity will be 1E+05 g^{-1}. Counting rules and stopping rules are specified in the method modification.

4.2.5.7 Quality Control for Tissue Burden Analysis

If tissue burden analyses are performed, the following QC requirements will apply.

Field-Based QC Samples

For LA tissue burden analyses, two types of field based QC samples well be collected.

T*issue Blanks* will be prepared at a rate of 2 blanks per day on days that tissues are processed. The tissue blank will contain a tissue sample of about 50 mg that does not have LA (e.g., liver from the supermarket) and is processed in the same manner as the other tissue samples. These blanks will identify if LA is introduced in the tissue sample collection and transportation processes.

Field Duplicates will be prepared at a target rate of one per day that tissues are processed. A field duplicate is a second piece of tissue derived from the same organ of the same animal. Because some tissues are small, and the minimum tissue mass for LA burden analysis is approximately 20 mg, field duplicates will usually not be possible for some of the smaller tissue types, but should be possible for some of the larger tissues from the larger animals.

Laboratory-Based QC Samples

Laboratory-based QC samples will be analyzed in accord with TEM ISO 10312 Method Modification 2, *Analysis of Tissue Samples for Asbestos by TEM* (see Attachment B). This method modification summarizes the acceptance criteria and corrective actions for TEM laboratory QC analyses that will be used to assess data quality.

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TECHNICAL MEMORANDUM

Date:	August 19, 2009
To:	Robert Marriam, Remedium Group, Inc.
From:	Sue Robinson, Parametrix Joe Volosin, Parametrix
Subject:	Small Mammal Reconnaissance Trip Report: Reference Area, Operable Unit 3, Libby Asbestos Superfund Site
cc:	Project Files
Project Number:	598-6068-001
Project Name:	Small Mammal Study, Remedial Investigation, Operable Unit 3 of the Libby Asbestos Superfund Site

INTRODUCTION

A reconnaissance trip to Libby OU3 and an upwind reference area was conducted between June 22 and June 24, 2009. In attendance were Sue Robinson, Carrie Claytor and Joe Volosin from Parametrix. The goals of the reconnaissance trip were to evaluate potential small mammal trap locations, determine the need (if any) for modifications to trapping methods and procedures as specified in the EPA Phase III Sampling and Analysis Plan (SAP), identify and mark the perimeter of the OU3 trap area, and establish terrain and trap area(s) accessibility within both the Libby Superfund Site, Operable Unit 3 (OU3) and the identified upwind (of OU3) reference area.

APPROACH

To help understand the sample area in OU3, the four soil/duff/tree bark sample points that define the corners of the small mammal sample area polygon were visited. The names and GPS coordinates for those sites are presented in Table 1. Additional locations throughout OU3 that can be used as trap locations were evaluated and the coordinates of these locations are also presented in Table 1.

As a potential reference area, the forest near Sheldon Mountain in the Kootenai National Forest was visited. The reference areas visited were all more than five miles (as the crow flies) from the SL15-02 location, which is the westernmost sample point in the OU3 small mammal sample area polygon. Coordinates for some of the key reference area locations visited are also presented in Table 1. Two potential reference locations, Ref. NW Point and Ref. East Point were originally derived from Google Earth to help guide finding the reference area.

OBSERVATIONS

The terrain in OU3 within the sampling polygon is very steep. The slope was also steep near each of the four corners of the sample area polygon. The terrain within the OU3 sample area generally included very dense shrubs and in places, dense tree growth. The OU3 locations where the terrain was not as steep were near sample areas, MOU301 and MOU302.

Table 1. OU3 and Reference Locations Evaluated during Reconnaissance Trip

Site Name	Easting	Northing	Location type
SL15-02	617648	5367516	OU3, Polygon corner point
SL45-02	618384	5367170	OU3, Polygon corner point
SL45-03	618801	5367750	OU3, Polygon corner point
SL75-03	619545	5366720	OU3, Polygon corner point
MOU301	619040	5367259	OU3, Additional point in area
MOU302	618467	5367604	OU3, Additional point in area
MOU303	617912	5367507	OU3, Additional point in area
MOU304	619522	5366709	OU3, Additional point in area
MOU305	617628	5367616	OU3, Additional point in area
MRFNW1	609230	5369918	Reference
MRFSW1	609048	5369563	Reference
MRFSW2	609124	5369703	Reference
MRFSW3	608667	5369099	Reference
MRFSW4	608459	5368977	Reference
MRFSW5	608398	5368861	Reference
MRFSW6	607835	5368438	Reference
MRFSW7	607871	5368657	Reference
MRFSW8	607540	5367894	Reference
MRFKW1	607256	5367451	Reference
MRFKW2	607183	5367290	Reference
MRFKW3	607253	5367240	Reference
MRFKW4	607350	5367434	Reference
Ref. NW Point	609242	5369986	Reference, GPS Estimated from Google Earth
Ref. East Point	610228	5369471	Reference, GPS Estimated from Google Earth

UTM NAD83, Zone 11

With the exception of locations adjacent to or in the immediate vicinity of roads and ranger-accessible paths, or where the lack of steep elevation permits deeper terrain access, much of the central area within the OU3 trap area polygon is terrain limiting and will not be trapped during the program. Trap locations established near vehicle access roads and pathways is important since field personnel must have ready access to vehicles for equipment storage and deployment and for efficient trap collection for transport back to the offsite processing laboratory.

The terrain near many of the potential upwind reference locations evaluated was also steep. The tree cover was generally greater at the reference locations than in OU3 but the shrub cover tended to be less dense. There is one potential reference area (near MRFKW2 and MRFKW4) that is not steep and is relatively open and that could be a possible backup reference location (i.e., should insufficient animals be caught at recommended reference areas). However, chipmunks and their burrows were observed in several locations throughout this backup area that would likely result in the capture of significant numbers of non-target species. Additionally, a Kootenai National Forest road (near FCC tower; road 4753A) was gated and locked which did not allow access to more forest land on Sheldon Mountain. The area behind this gate is another location that may be desirable for trapping and Parametrix is requesting that Remedium discuss gaining access with the Kootenai National Forest.

Finally, terrain limiting conditions (steepness) will also affect the placement, number and spacing of traps in both reference and OU3 areas. The Phase III SAP specified distance of 100 meters is simply considered unrealistic for the nature of the terrain conditions in both OU3 and the upwind reference areas.

RECOMMENDATIONS

It will not be possible to have complete coverage within the small mammal sample area polygon in OU3. The steepness of the terrain and the shrub density will hinder travel across the small mammal sample area. To implement the sample program, the sample locations will have to follow the logging roads within OU3 (Figure 1). General small mammal collection areas that will be used include MOU301 through MOU305 as well as the soil sample points[1], SL15-02, SL45-02, SL45-03 and SL75-02. Trap-lines will be set up on both sides of roads in these distinct locations spread throughout the small mammal sample area polygon.

Similarly, for the reference area, the trap-lines will be set up on both sides of forest roads. The trap lines will be set at distinct locations spread throughout the reference area (Figures 2 and 3).

As previously discussed, due to the density of the shrubs and steepness of the terrain the distance between traps in both the reference and OU3 locations will have to be much closer than the 100 meters specified in the Phase III SAP. A distance of 10 meters (33 feet) is recommended and considered more realistic for the terrain conditions. This distance will be appropriate for ensuring small mammal collection but would not be adequate for attempting to trap separate populations with each and every trap (Bowman et al. 2000), which is probably not necessary to meet the goals of this sampling program. However, the goal to keep the reference and OU3 populations separate will be achieved as the sample areas are more than five miles apart. It is noted that Pearson and Ruggiero (2003) had good results capturing deer mice (*Peromyscus maniculatus*) and southern red-backed voles (*Clethrionomys gapperi*) in west-central Montana when traps were set 10 meters apart using a transect method. Therefore, traps will be spaced at an interval appropriate to field terrain conditions but no closer than 15 feet apart.

In each sample area (e.g., OU3), 100 traps will be used but not more than 40 – 50 traps will be set each night. This number of traps will make trap set-up and retrieval manageable given the need to set traps close to dusk and retrieve early in the morning.

REFERENCES

Bowman, J., Forbes, G. and Dilworth, T. 2000. The spatial scale of variability in small-mammal populations. Ecography 23: 328–334.

Pearson, D.E and L. F. Ruggiero. 2003. Transect versus grid trapping arrangements for sampling small-mammal communities. Wildlife Society Bulletin, Vol. 31, No. 2, pp. 454-459.

[1] Each of the four points that made the polygon were near a haul road or logging road.

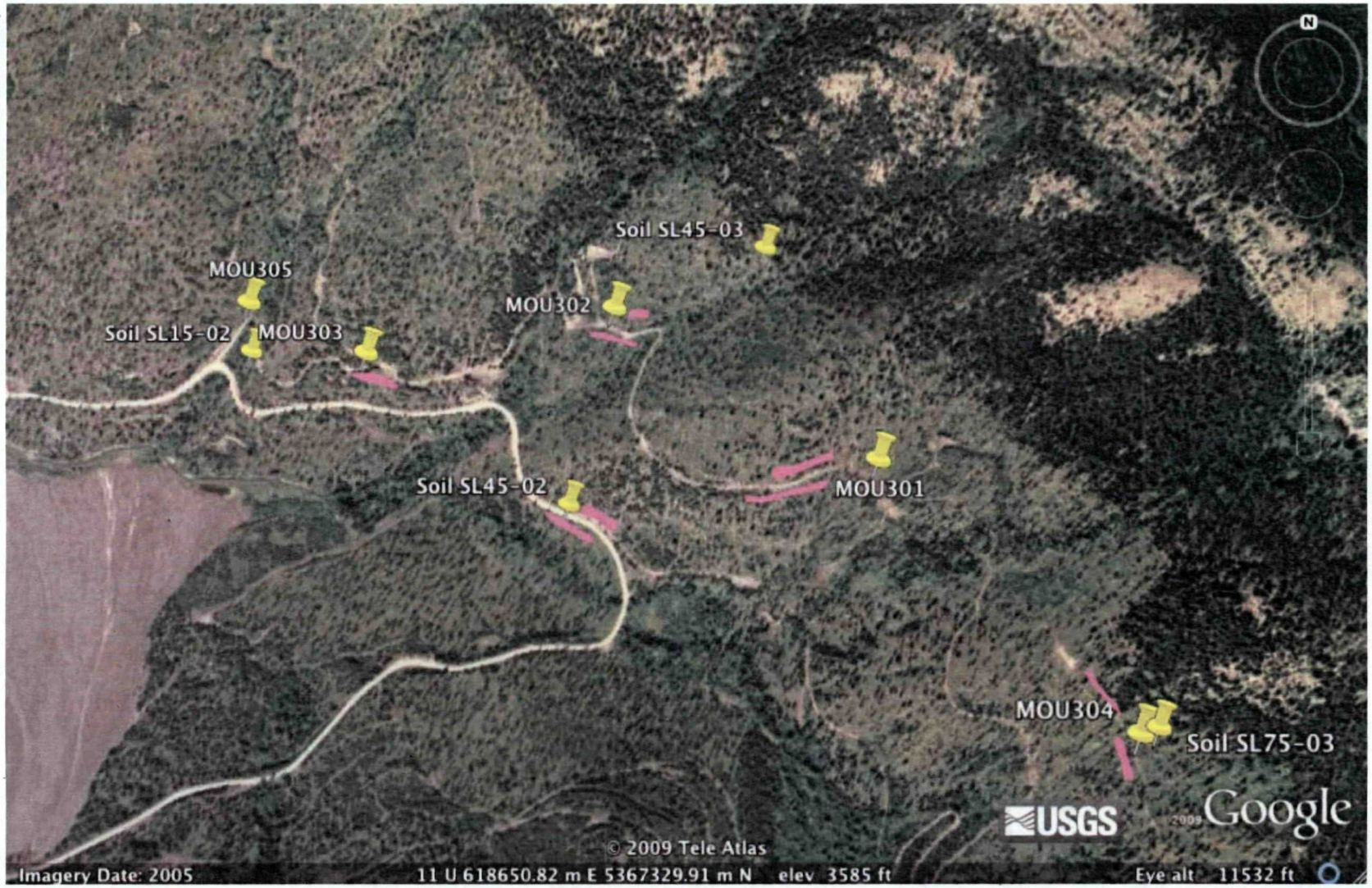

Figure 1. Small Mammal Sample Area in Libby Superfund Site, Operable Unit 3

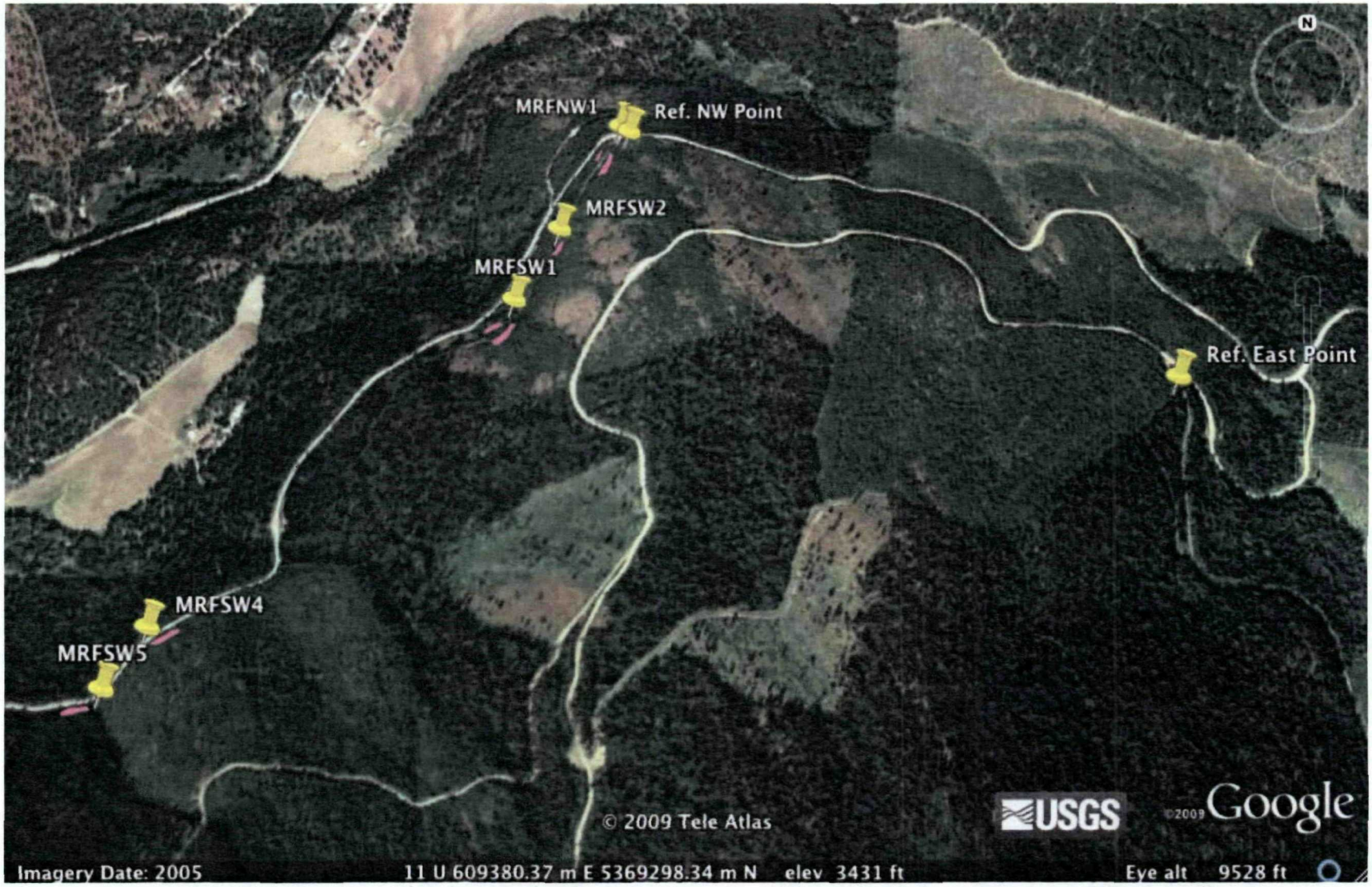

Figure 3. Small Mammal Sample Area, Reference Area 2

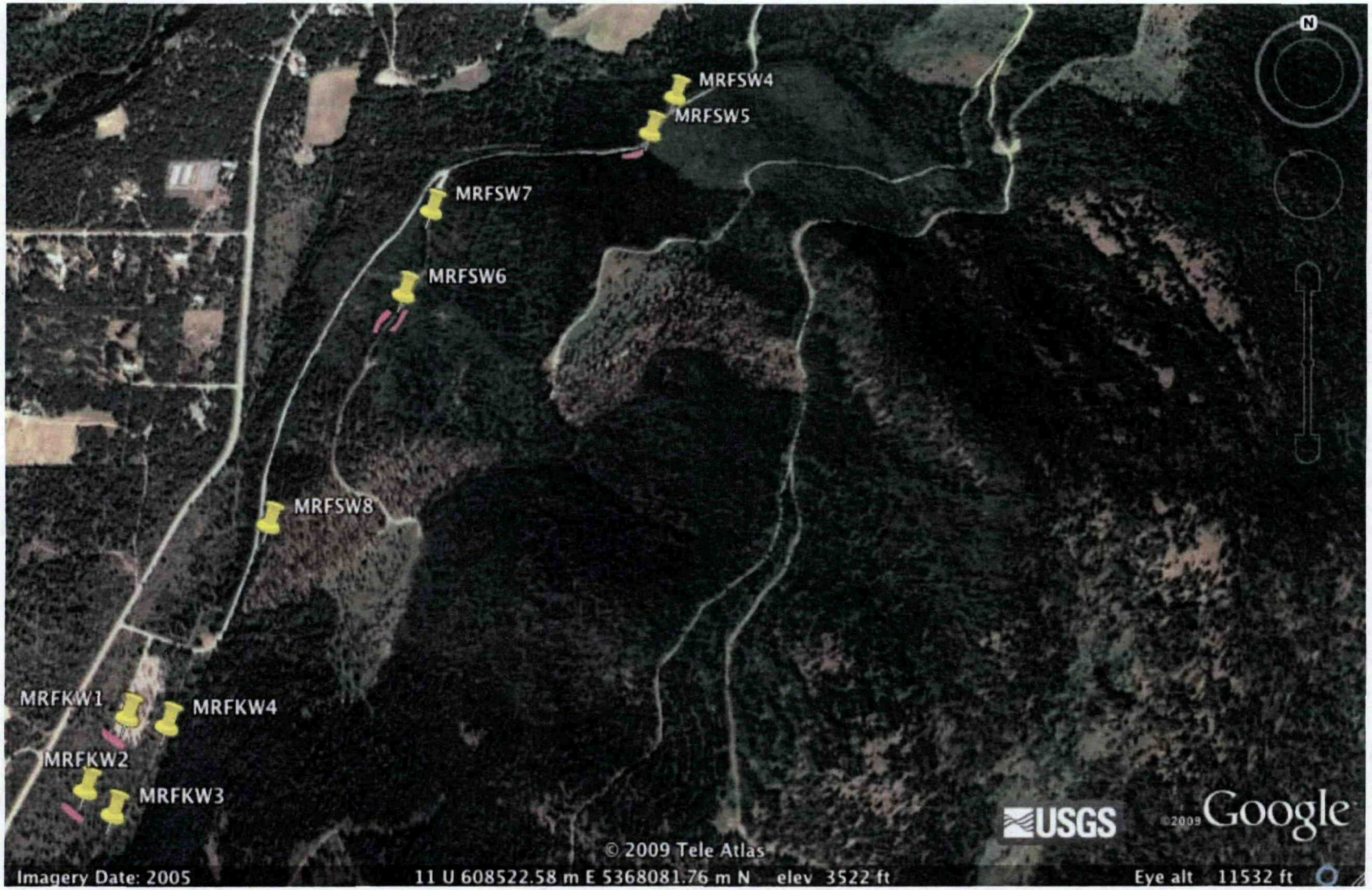

Figure 2. Small Mammal Sample Area, Reference Area 1